Contemporary Formulation Development for Inhaled Pharmaceuticals

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Abstract

Pulmonary delivery has gained increased interests over the past few decades. For respiratory conditions, targeted drug delivery directly to the site of action can achieve a high local concentration for efficacy with reduced systemic exposure and adverse effects. For systemic conditions, the unique physiology of the lung evolutionarily designed for rapid gaseous exchange presents an entry route for systemic drug delivery. Although the development of inhaled formulations has come a long way over the last few decades, many aspects of it remain to be elucidated. In particular, a reliable and well-understood method for in vitro-in vivo correlations remains to be established. With the rapid and ongoing advancement of technology, there is much potential to better utilise computational methods including different types of modelling and simulation approaches to support inhaled formulation development. This review intends to provide an introduction on some fundamental concepts in pulmonary drug delivery and inhaled formulation development followed by discussions on some challenges and opportunities in the translation of inhaled pharmaceuticals from preclinical studies to clinical development. The review concludes with some recent advancements in modelling and simulation approaches that could play an increasingly important role in modern formulation development of inhaled pharmaceuticals.

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Introduction

For physiological conditions within the respiratory tract, targeted delivery of the drug directly to the site of action could provide benefits such as achieving a greater local concentration at the target site with a reduced dose, resulting in reduced systemic exposures and adverse events.1 Most commercial products of inhaled formulations are developed for the treatment of local conditions or diseases active in the respiratory tract. Inhaled corticosteroids, long-acting beta-agonists, short-acting beta-agonists and long-acting muscarinic antagonists have long been used for the management of asthma and chronic obstructive pulmonary disease (COPD). Inhaled mannitol and antibiotics, to a lesser extent, have been used in the management of cystic fibrosis (CF).2–4

Abbreviations: ACI, Andersen Cascade Impactor; AIM, Abbreviated impactor measurement; AM, Alveolar macrophages; ANN, Artificial neural network; API, Active pharmaceutical ingredient; APSD, Aerodynamic particle size distribution; AT-I, Alveolar cells type-I; AT-II, Alveolar cells type-II; BALF, Bronchoalveolar lavage fluid; BCS, Biopharmaceutical Classification System; CF, Cystic fibrosis; CFC, Chlorofluorocarbon propellants; CFD, Computational fluid dynamics; COPD, Chronic obstructive pulmonary disease; DC, Dendritic cell; DPI, Dry powder inhaler; EBC, Exhaled breath condensate; ECG, Enhanced condensational growth; EEG, Excipient enhanced growth; ELF, Epithelium lining fluid; FTH, First-time-in-human; FPD, Fine particle dose; FPF, Fine particle fraction; FSA, Fast Screening Andersen; GI, Gastrointestinal; GRAS, generally recognised as safe; IBCS, Inhalation Biopharmaceutical Classification System; IPL, Isolated perfused lung; HFA, Hydrofluoroalkane propellants; IP, Induction port; IVIVC, In vitro-in vivo correlation; MC, Mast cell; MCC, Mucociliary clearance; MD, Molecular dynamics; MDI, Metered-dose inhaler; MMAD, Mass median aerodynamic diameter; MPPD, Multiple-path particle dosimetry; NEB, Nebuliser; NGL, Next Generation Impactor; NLME, Non-linear mixed effects; PBPK, Physiologically-based pharmacokinetics; PK, Pharmacokinetics; PD, Pharmacodynamics; PET, Positron emission tomography; PLGA, Poly(-lactide-co-glycolide); pMDI, Pressurised metered-dose inhaler; PSD, Particle size distribution; rNGI, Reduced Next Generation Impactor; SPECT, Single photon emission computed tomography; TSI, Twin-stage impinger; USP, United States Pharmacopeia.

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In recent years, systemic drug delivery through pulmonary administration has gained increased interest. The unique physiology and high absorptive capacity of the lung resulting from the large surface area, the thin epithelial lining and the high permeability physiologically designed for rapid gaseous exchange, provide a potential entry route for compounds that are not orally bioavailable. In particular, pulmonary administration avoids the barriers limiting drug absorption in the gut, allowing the systemic delivery of not only large biopharmaceuticals (e.g. proteins and peptides) but also small molecules where oral bioavailability is limited by first-pass metabolism and efflux transporters. For instance, the use of nebulised fentanyl as an alternative pain relief option in emergency settings has been reported to provide more rapid and sustained pain relief compared to intravenous morphine. The approval of inhaled insulin has demonstrated the feasibility of systemic delivery of biopharmaceuticals via pulmonary administration. These are examples showing the potential of the pulmonary system as a non-invasive option for systemic delivery of a variety of therapeutic classes including small molecules as well as biopharmaceuticals such as antibodies, vaccines and deoxyribonucleic acids (DNAs), instead of the more invasive parenteral route conventionally required for the delivery of these agents.

There is clearly an increasing interest in pulmonary drug delivery over the last few decades. As an indication, a simple PubMed search using the term “inhaled formulations” yielded almost >2300 results for the time period 2010–2019, while the same search yielded <1350 results for the previous decade (2000–2009) and <420 results for the 10-year period until 1999. However, compared to more conventional routes of administration such as the oral and parenteral routes, inhaled drug delivery presents its unique set of formulation challenges and considerations such as careful control of particle size and aerosolisation are crucial. Despite the many advances in the field over the last few decades, many aspects of inhaled drug delivery remain poorly defined and efforts to demystify some of these areas continue to be an ongoing endeavour. In particular, a well-validated model for the investigation of in vitro-in vivo correlation (IVIVC) of inhaled formulations remains to be established. With the rapid advancement in technology and computing power, there is much potential to better utilise various computational methods to facilitate drug discovery and development. In recent years, efforts to more widely apply different modelling and simulation approaches in the area of inhaled pharmaceuticals have started to emerge.

This review serves two main purposes: (i) to provide an overview on some fundamental concepts in pulmonary drug delivery and inhaled formulation development and (ii) to present and discuss challenges and opportunities in the translation of inhaled pharmaceuticals from preclinical studies to clinical development. Hence, this work has condensed some classical concepts from the literature to summarise the key considerations in the development of inhaled formulations. Building on these fundamentals, recent advancements in this space in particular the emergence of various modelling and simulation methodologies applicable to the development of inhaled drug products are presented. This review concludes with the potential roles of these modelling and simulation approaches at different stages of the development process in modern drug discovery and development of inhaled pharmaceuticals.

**Physiology of the Respiratory System**

**Basic Structure of the Respiratory Tract**

The respiratory system consists of a number of components involved in breathing including nose, pharynx, larynx, trachea, bronchi and lungs. The respiratory system can be functionally divided into two parts, namely the conducting zone and the respiratory zone. The conducting zone, comprising the upper airways from the trachea to the terminal bronchioles, is largely composed of varying degrees of cartilages and mucociliary clearance (MCC) is prominent in this region. The respiratory zone, comprising the respiratory bronchioles, alveolar ducts and alveoli, is physiologically designed to facilitate rapid gaseous exchange. The right lung is composed of three lobes while the left lung has only two lobes. Upon inspiration, air enters via either the nose or the mouth and then travels down the throat through the larynx and trachea before reaching the lungs through the two main-stem bronchi reaching the left and right lung. In the lungs, the main-stem bronchi divide into branches of smaller bronchi and even smaller bronchioles before reaching the terminal bronchioles and alveoli, where gaseous exchange takes place as oxygen diffuses through the alveolar wall into the blood in exchange of carbon dioxide.

The branching of the airways serves to provide a sifting mechanism to trap inhaled particles via inertial impaction and sedimentation while humidifying and warming inhaled gases to body temperature. The tracheobronchial tree bifurcates repeatedly creating up to 23 divisions — including 16 divisions within the conducting zone and 7 within the respiratory zone — before reaching the alveoli (Table 1). The airflow velocity decreases from 150 cm/s in the upper conducting airways to nearly zero in the alveolar region. There is a reduction in diameter and length of the bronchi with a concomitant increase in their number and surface area with each division of the tracheobronchial tree. The amount of smooth muscles and cartilage as well as the thickness of the airway walls also decrease with each division. Eventually, there is no smooth muscles and cartilage and only thin alveolar epithelium in the peripheral lung. The combination of large alveolar surface area (>100 m²) and small diffusion distance (approx. 1 μm) in the respiratory zone not only facilitates efficient gaseous exchange but also systemic drug delivery via the pulmonary route.

**Physiological Barriers and Clearance Mechanisms**

In the conducting airways, deposited particles are removed relatively quickly by MCC, the primary mode of defence of the respiratory system, with a half-life of approximately 1.5 h. The conducting airways are lined by epithelium consisting primarily of two types of cells — i.e. mucus-producing goblet cells (20%) and ciliated cells (80%), which comprises the “mucociliary escalator”. Inhaled particulates and infectious debris are trapped by the mucus blanket produced by the goblet cells and is in turn propelled and transported by the ciliated cells to the gastrointestinal (GI) tract. The continuous transport of mucus towards the proximal trachea and oesophagus limits any accumulation of particles within the airway. The characteristics of the lung lining fluid differ at various parts of the airways and transition from predominantly mucins in the conducting zone to predominantly surfactants in the alveoli. Within the alveoli, pulmonary surfactants form a mono-layer on the alveolar lining fluid. It consists primarily of phospholipids but also other specific surfactant proteins. Surfactants facilitate clearance of particles in the lung by dispersion and adsorption. The characteristics of the lung lining fluids including the composition and approximate layer thickness are summarised in Table 2.

The cellular morphology of the pulmonary mucosa along the respiratory tract changes significantly in accordance with their physiological functions. The epithelial cells in the conducting airways are pseudostratified, columnar and ciliated. The
epithelial cells transition to a cuboidal form deeper down in the bronchial tree to the alveoli. Two prominent types of epithelial cells can be found in the alveoli, i.e. alveolar cells type I (AT-I) and type II (AT-II). The alveolar epithelium is sealed by tight junctions between these cells against the entrance of foreign material and pathogens forming an “air-blood barrier”. The flat and squamous AT-I cells cover >90% of the surface area. They are therefore the major cell type of the alveolar epithelial lining, forming the thin and permeable barrier for rapid gaseous exchange. The small and cuboidal AT-II cells are dispersed throughout the alveoli between the AT-I cells and they synthesise, secrete and recycle all surfactants that regulate alveolar surface tensions. While AT-I cells are considered terminally differentiated and unable to perform further division, AT-II cells are considered to be renewing cells. AT-II cells differentiate into AT-I cells and are the progenitors for both types of alveolar cells. AT-II cells also act as immunoregulatory cells as they produce a wide range of pro-inflammatory mediators including various cytokines and chemokines.

In the alveolar region, the clearance of particles and pathogens are predominantly performed by alveolar macrophages (AMs) due to the lack of mucociliary escalator. It is estimated that over 2.3 billion of these cells are present throughout the lungs in an healthy individual. AMs are the principal phagocytic and scavenger cells in alveoli and account for up to 90% of immune cells in the alveolar spaces. These cells engulf particulates, process antigens and kill ingested microorganisms by phagocytosis. In addition to their phagocytic function, AMs also play a prominent role in lung immunity by initiating inflammatory and immune responses. Activated AMs secrete pro-inflammatory mediators such as various chemokines and cytokines to recruit other immune cells. AMs show size-dependent uptake and are most effective for particles with a geometric diameter of 0.5–5 μm. After phagocytosis, AMs travel along the alveolar surface to the mucociliary escalator for clearance. Other immune cells including dendritic cells (DCs) and mast cells (MCs) also play a role in lung immunity and their functions have been nicely reviewed in the literature and should be referred to for further details.

### Regional Drug Delivery within the Respiratory Tract

The regional control of particle deposition is one of the specific challenges for inhaled drug delivery. The efficacy of the formulated drug is highly dependent on its deposition pattern within the respiratory tract. Given the different physiological barriers, it is not surprising that formulations deposited in different regions of the respiratory tract are absorbed differently. For dry powder formulations, solid materials deposited in different regions of the lungs are subjected to different clearance mechanisms resulting in different residence time. In a sheep study, it was shown that particles that were deposited in the upper airway could be cleared within 2–4 h, while particles deposited in the distal bronchi and alveolar region could remain as long as 72 h. Therefore, it is clear

| Zone | Generation | D (cm) | L (cm) | N | Cross-Section Area (cm²) | Cartilage | Epithelial Cell Type |
|------|------------|--------|--------|---|--------------------------|-----------|---------------------|
| Conducting zone | Trachea | 0 | 1.8 | 12.0 | 1 | 2.54 | Open rings | Columnar ciliated |
| | Bronchi | 1 | 1.22 | 4.8 | 2 | 2.33 | Plates |
| | 2 | 0.83 | 1.9 | 4 | 2.13 | Plates |
| | 3 | 0.56 | 0.8 | 8 | 2.00 | Plates |
| | Bronchioles | 4 | 0.45 | 1.3 | 16 | 2.48 | Plates |
| | Terminal bronchioles | 5 | 0.35 | 1.07 | 32 | 3.11 | Plates |
| Respiratory zone | Respiratory bronchioles | 16 | 0.06 | 0.17 | 6 x 10⁴ | 180.0 | Absent | Cuboidal to alveolar |
| | 17 | 0.17 | 0.42 | 5 x 10⁵ | 103 | Cuboidal |
| | 18 | 0.56 | 0.83 | 8 | 2.00 | Plates |
| Alveolar ducts | 19 | 0.05 | 0.10 | 5 x 10⁵ | 103 | Alveolar |
| | 20 | 0.17 | 0.34 | 5 x 10⁵ | 103 | Alveolar |
| | 21 | 0.34 | 0.67 | 5 x 10⁵ | 103 | Alveolar |
| | 22 | 0.67 | 1.34 | 5 x 10⁵ | 103 | Alveolar |
| Alveolar sacs | 23 | 0.03 | 0.03 | 8 x 10⁶ | 104 | Alveolar |

Abbreviations: D – diameter; L – length; N – number of airway.

| Table 2 | Characteristics of the Human Lung Lining Fluid in the Conducting Airways and the Respiratory Zone |
|---------|-----------------------------------------------------------------------------------------------|
| Properties | Conducting Airways | Respiratory Zone |
| Principal lining fluid Composition | Mucous | Surfactant |
| | 1% inorganic salts | 85% phospholipids |
| | 1% proteins | 5% cholesterol |
| | 2% glycoproteins (mucins) | 10% surfactant proteins (e.g. SP-A, SP-B, SP-C, SP-D) |
| 99% water | 95% water |
| Layer thickness | 3–15 μm (decrease in thickness in lower airways) | -0.07 μm |
| Approximate volume | 10–30 mL | 7–20 mL |
that different deposition patterns can result in different pharmacokinetic (PK) profiles, and subsequently different pharmacological effects, both locally and systemically.

For solution formulations, once delivered and deposited, the absorption of the drug is primarily dependent on the physicochemical properties of the drug molecule and the physiological barriers at the deposited region. For dry powder formulations, the deposited solid particles also have to survive the local defence and clearance mechanisms during dissolution. In the context of pulmonary drug delivery, the respiratory tract can be broadly divided into three deposition regions which in turn determine the fate of the deposited particles. In the oropharynx region, deposited particles are swallowed into the GI tract. In the conducting airways, deposited particles are cleared by MCC and eventually swallowed. In the respiratory zone, deposited particles are primarily cleared by cellular mechanisms such as phagocytosis by alveolar macrophages (Fig. 1).30

The optimal delivery region depends on the intended therapeutic purpose and the disease condition. For local treatments, e.g. asthma, COPD and respiratory infections, high lung concentrations are favourable for maximal local efficacy and reduced systemic side effects. Conversely, maximum absorption and bioavailability is desirable for systemic delivery of biopharmaceuticals, e.g. insulin and vaccines. The fate of inhaled particles, and hence the absorption of the drug, is determined by their distribution pattern in the lungs. Therefore, a good understanding and control of particle deposition, from the device to the airways, is needed for rational design and development of inhaled pharmaceuticals.

**Particle Characteristics and Deposition Pattern**

**The Importance of Particle Deposition Pattern**

In the context of inhaled drug delivery, particle deposition pattern concerns not only the distribution of the drug throughout the airways, but also the amount retained in the device, deposited at the oropharyngeal region, and exhaled following administration. Even for the same formulation and aerosolisation conditions, the relationship between the filled mass of the formulation and the resulting fine particle dose (FPD) is not straightforward. This unique challenge is reflected by the complex dose equivalence observed in the development space of inhaled insulin.31 For Exubera®, the nominal doses of 1 and 3 mg are equivalent to 3 and 8 units of insulin, respectively. This non-linearity in dose adjustment was a result of the different aerosolisation and delivery efficiency of the 1 and 3 mg blisters. For the 1 mg blister, a fill mass of 1.7 mg powder formulation in the capsule was needed for an emitted dose of 0.53 mg insulin and a FPD of 0.4 mg insulin, whereas for the 3 mg blister, a fill mass of 5.1 mg powder formulation was needed for an emitted dose of 2.03 mg insulin and a FPD of 1.0 mg insulin.32 In contrast, for the AIR® insulin delivery system, the different combinations of the capsules have been shown to be interchangeable.33 Product testings need to take into account these considerations appropriately. Particle deposition pattern is a function of a number of factors including the particle size distribution (PSD) and the aerosolisation efficiency of the formulation. The understanding of the relationship between particle characteristics and deposition pattern of inhaled formulations are therefore of critical importance.
Particle Size and Aerodynamic Diameter

The control of particle size is critical in inhaled drug delivery. In principle, for particles of unit density, it is generally recognised that particles with diameters of >5 μm largely deposit in the mouth and upper airways and are unlikely to reach the deep lung, while delivery to the lower airways requires particle diameters in the range of 1–5 μm for efficient deep lung deposition. Particles smaller than 1 μm in diameter have long been suggested to deposit less efficiently as they are predominantly exhaled. Recently, it has been suggested that very small ultrafine particles with diameters <100 nm may be able to enter the bloodstream directly by translocation from the respiratory system, although the exact mechanism remains unclear. Hence, it is clear that the change in particle size distribution can impact on the deposition pattern and clinical efficacy of the formulated drug. The relationship between particle size and deposition pattern has been widely studied and discussed in the literature.

The mode of delivery and the choice of device are important factors controlling particle size and shape. The various considerations for different devices are discussed in Section Delivery Systems, Formulations and Devices. The types of aerosol delivery systems can be broadly divided by the physical state of the aerosolised particles as either a liquid or solid aerosol delivery system. For liquid-based systems, droplet size is largely dependent on the atomising efficiency of the device. For instance, effective nebulisation, and hence droplet size distribution, is largely dependent on the design and efficiency of the nebuliser. For pressurised metered-dose inhalers, in addition to the design of various device components (e.g. valve and nozzle), the choice of the propellant system is also important. For solid-based systems, the dispersibility and deagglomeration of particles provide an additional layer of complexity in formulation development. The control of PSD in the appropriate range is a prerequisite in optimising particle deposition pattern within the respiratory tract.

While PSD is undoubtedly important, it is not the sole factor dictating the aerodynamic behaviour of particles. For instance, porous particles of low density can travel further in the respiratory tract than non-porous particles of the same geometric size. Therefore, aerodynamic diameter, which is the diameter of a unit-density sphere that has the same settling velocity as the measured particle, provides a more relevant description of particle size for respiratory delivery. The relationship between aerodynamic diameter (\(d_a\)), geometric diameter (\(d_g\)) and particle density (\(\rho_p\)) is shown in Equation (1).

\[
d_a = d_g \sqrt{\frac{\rho_p}{\rho}}
\]

The equation is a simplified version using the reference density of a spherical calibration particle (\(\rho\)) (i.e. 1.0 g/cm³). The correction of the equation for non-spherical particles has been described in the literature and is beyond the scope of this review. Interested readers are referred to publications, for instance, by Carvalho et al. and Shekunov et al. To account for the aerodynamic properties of particles, instead of geometric diameter, mass median aerodynamic diameter (MMAD) is more commonly used as a more relevant measurement for inhaled formulations.

Aerosolisation

The aerosolisation efficiency of a formulation is critical in inhaled drug delivery. Ultimately, it is the aerosolisation performance that determines the particle deposition pattern. The in vitro aerosolisation performance of a formulation is therefore commonly used as an indicator of its delivery efficiency. Aerosolisation is generally achieved by producing particles of sizes in the respiratory range (i.e. 1-10 μm). For liquid formulations, however, droplet size may change when travelling through the respiratory tract due to, for instance, the high relative humidity in the lung environment. Therefore, particles may have a different deposition pattern compared to what is expected from the original MMAD of the formulations. While not a common approach yet, it should be noted that intentional size increase of aerosolised particles upon inhalation using methods such as enhanced condensational growth (ECG) and excipient enhanced growth (EEG) has been studied as an approach to increasing deposition of submicron size particles or targeting deposition in the tracheobronchial airways. Hence, it is important that such changes in particle size upon inhalation, and its subsequent implications on particle deposition, are considered during formulation development.

For dry powder formulations, sufficient powder dispersibility and de-agglomeration is crucial for efficient aerosolisation. It is well-established that inter-particulate cohesive forces increase with decreasing particle size and are particularly dominant between small particles. Hence, these forces play an important role in the particle size range required for efficient pulmonary delivery. When agglomerates are formed due to poor powder dispersion, the material behave aerodynamically as large particles with poor aerosolisation performance. Therefore, formulation strategies that can result in highly dispersible powders such as the inclusion of suitable excipients to increase dispersibility and careful particle engineering have received much interest. For instance, spray-drying and spray-freeze-drying have been used extensively to produce highly aerosolisable multi-component formulations with multiple functionalities. These “smart formulations” were carefully designed and engineered to have, in addition to being aerosolisable, multiple capabilities such as stabilising the active compounds or providing a controlled-release profile.

Mechanisms of Particle Deposition

Deposition of inhaled particles in the respiratory tract occurs via a number of mechanisms including inertial impaction, sedimentation, diffusion, interception and electrostatic interactions (Fig. 2). Depending on the physical properties of the particles (e.g. size, mass, density and shape), some of these mechanisms are more influential than others. In general, inertial impaction is the most dominant mechanism for deposition of large particles in the upper airway with high flow velocity, when the momentum of the particle is too large for it to follow the rapid change in directions of the bulk airstream. Sedimentation is driven by gravity and is a function of particle size, density and residence time in the airway, while diffusion is the primary mechanism for deposition of submicron size particles where Brownian motion dominates.

The morphology of particles also appears to be a factor influencing particle deposition. In one study, spherical spray-dried particles produced significantly higher fine particle fractions (FPFs) for deep lung deposition compared to angular jet-milled particles. Fibrous particles, for instance, are more likely to be deposited by interception, which occurs as the particle contacts and is subsequently retained by the surrounding surface of an airway, even though the centre of mass of the particle remains on a fluid streamline. Electrostatic charges have been suggested to increase mouth-throat deposition of very fine particles (i.e. <0.1 μm). It may also increase deposition in upper airways by increasing agglomeration, and therefore the aerodynamic size, of small particles. Other factors such as posture and inhalation flow rate have also been shown to impact on particle deposition.
Breathing Pattern and Disease Status

The importance of particle deposition pattern means that inhaled drug delivery is susceptible to the natural variability in breathing. The inspiratory energy available for aerosolisation of a formulation and transportation of particles throughout the tracheobronchial tree is dictated by a complex set of breathing parameters. Breathing frequency and tidal volume can affect the lung residence time of aerosolised particles, and hence the probability of deposition. Similarly, flow rate dictates the degree and extent of turbulence that promote particle deposition in upper airways. In a study using a jet nebuliser, it has been shown in human adults that, compared to their own breathing frequency (16 ± 5 breaths per minute), slower guided breathing (11 breaths per minute) increased pulmonary deposition significantly. In another study, the significance of breath-hold time was studied in COPD patients. The study compared the lung dose of six commercial inhalers after no breath-hold and a breath-hold of 5 s and 25 s. The lung dose was enhanced by as much as 26% and 53% after a breath-hold of 5 s and 25 s, respectively. Hence, a change in breathing profile can change the lung deposition pattern, and subsequently the absorption and effect of the drug.

One of the unique challenges in inhaled drug delivery is the breath-to-breath variability in inspiratory effort leading to high between and within subject variability. This intrinsic variability needs to be carefully considered, especially for systemic delivery of drugs with narrow therapeutic windows such as potent bio-pharmaceuticals, as it translates to variable bioavailability inherent to this route of administration. In addition, it also means that populations with different inspiratory efforts, such as patients of different age groups and disease status, will require special considerations. Airway conditions are typically characterised by airway narrowing and mucus accumulation, which could impact on the lung distribution of inhaled formulations. While lung deposition is usually higher in patients with obstructive airway diseases (e.g. asthma and COPD) compared to that of healthy subjects, there is also a shift in deposition pattern towards the central lung in the presence of bronchoconstriction with a corresponding decrease in peripheral lung deposition. In addition, it has also been shown that total deposition is higher in CF patients compared to that in normal subjects. It should be noted that, given the complexity of airway diseases, deposition patterns are also more heterogeneous in diseased lungs than in healthy lungs. It is therefore advantageous to develop a high-performance formulation with consistent aerodynamic behaviours across a wide range of flow rates to mitigate the dependency on respiratory efforts.

Mode of Delivery, Formulation and Device

Mode of Delivery

The three principal categories of delivery systems used for the administration of inhaled therapies are nebulisers (NEBs), pressurised metered-dose inhalers (pMDIs) and dry powder inhalers (DPIs). These systems produce small particles in the inhalation range for respiratory drug delivery via different mechanisms, and each of them has its respective characteristics. NEBs operate by atomising the bulk liquid formulation into fine droplets for inhalation. It can be perceived as relatively simple to formulate a drug for nebulisation since virtually any drug in a liquid formulation, given the appropriate device and conditions, can be delivered at almost any dose by NEBs. However, traditional NEBs are usually cumbersome and inconvenient to carry and operate. Some well-documented disadvantages include high cost, low efficiency, poor reproducibility and high variability, risk of bacterial contamination and constant cleaning requirements. Their use can be time-consuming, often requires a power supply and may be quite noisy during administration. Depending on the drug, it can take as long as 30 min for delivery if set-up, drug administration and cleaning are taken into account. In recent years, smaller and more portable NEBs have been developed to address these issues. MicroAir® and I-neb® are notable examples that are small enough to be carried in a standard handbag with more portable battery powering mechanisms compared to traditional NEBs.

In contrast, pMDIs provide a more convenient and portable treatment alternative compared to NEBs for the delivery of liquid formulations, with reduction in treatment preparation and administration time, resulting in a potential reduction in medication cost and healthcare resources. Since the introduction in the 1950s, pMDIs remain well-accepted and highly utilised in the management of asthma and COPD. The effective use of pMDIs, however, requires specific breathing technique that involves adequate coordination between inspiration and actuation of the inhalers. It has been reported that many patients and healthcare
Table 3
Examples of Investigational Excipients Reported in the Literature for Pulmonary Delivery.

| Class          | Excipient | Range (FDA) | Form | Comment |
|----------------|-----------|-------------|------|---------|
| Amino acids    | Leucine   | 10% w/w     | DPI  | Improves aerosolisation of the SD powder |
|                | Glycine   | 12.3% w/w (2 mg) | DPI  | Glass stabiliser in the lyophilised formulation |
|                | Alanine   | 40–55% w/w  | DPI  | Crystalisation inhibitor in the SD formulation |
|                | Methionine| <5% w/w     | DPI  | Potential adjunct therapy for PA infection |
|                | Tryptophan| <5% w/w     | DPI  | Potential adjunct therapy for PA infection |
|                | Tyrosine  | <3% w/w     | DPI  | Potential adjunct therapy for PA infection |
| Small carbohydrates | Lactose | <98.5% w/w (13 mg) | DPI  | Coarse carrier particles in blended formulation |
|                | Mannitol  | 30–100% w/w (6 mg) | DPI  | Glass stabiliser in the SD formulation |
|                | Trehalose  | <90% w/w    | DPI  | Glass stabiliser in the SD formulation |
|                | Sucrose   | <11.5% w/v  | NEB  | Viscosity enhancer |
| Polysaccharides| Dextran   | <30% w/w    | DPI  | Glass stabiliser in the SD formulation |
|                | HA        | 5–10% w/w   | DPI  | Crystalisation inhibitor in the SD formulation |
|                | Chitosan  | NA          | DPI  | Mucocoadhesive agent |
| Synthetic polymers| PVP K25  | 75% w/w     | DPI  | Glass stabiliser in the lyophilised formulation |
|                | PVP K30   | 5% w/w      | DPI  | Coating of lactose to modify carrier function to enhance API particle liberation and aerosol performance |
|                | PVP K30   | 0.0075% w/w | MDI  | Surface active polymer used as suspension stabiliser |
|                | EC        | 5% w/w      | DPI  | Coating of lactose to modify carrier function to enhance API particle liberation and aerosol performance |
|                | PS 20     | ≤0.01% w/v  | NEB  | Surface active polymer used as a stabiliser to prevent protein aggregation |
|                | PS 80     | ≤0.01% w/v (0.02% w/v) | NEB  | Surface active polymer used as a stabiliser to prevent protein aggregation |
|                | PX 188    | <20% w/v    | NEB  | Surface active polymer used as suspension stabiliser |
|                | Solutol®  | <20% w/v    | NEB  | Surface active polymer used as suspension stabiliser |
|                | PEG 300   | 0.075% w/w  | MDI  | Steric stabiliser for the suspension |
|                | PEG (200, 400 and 600) | 0.55% w/v | MDI  | Steric stabiliser for the suspension |
|                | PLGA      | NA          | DPI  | Carrier for prolonged release of active |
|                | NaCMC     | 99.8% w/w   | DPI  | Carrier in the SD formulation |
|                | Starch    | 99.8% w/w   | DPI  | Carrier in the SD formulation |
| Surfactants    | Brij-35   | ≤0.01% w/v  | NEB  | Stabiliser to prevent protein aggregation in solution |
|                | SorbMO    | 0.13% w/w   | MDI  | Stabilising agent |
| Phospholipids  | DPPC      | 0.5–20% w/v | DPI  | Self-assemble into porous microparticles |
| Miscellaneous  | FDKP      | <90% w/v    | DPI  | Solubilising and CR agent |
|                | CD        | ≤30% w/v    | NEB  | Solubilising and CR agent |
|                | AB        | NA          | DPI  | Process enhancer as a pore-forming agent, which decomposes at 36–60 °C into ammonia, carbon dioxide and water vapour |
|                | NaCl      | 50–75% w/v  | DPI  | Hygroscopic agent for EGG delivery in CFD model |
|                | NaCl      | 0.0048% w/v (23 mg) | NEB  | Electrolyte for conductivity to reduce the effect of surface charge leading to smaller atomised droplet size |
|                | NaCit     | 4–45% w/v (17 mg) | DPI  | Crystalisation inhibitor in the SD formulation |
|                | NaAlg     | 30% w/v     | DPI  | Slow drug transport across Calu-3 cell layers |
|                | Glycerol  | 0.35% w/v   | MDI  | Solubilising agent |
|                | Ethanol   | 15% w/w     | MDI  | Solubilising agent |

Abbreviations: AB = ammonium bicarbonate; CD = cyclodextrin; CFD = Computational fluid dynamics; CR = controlled-release; DPI = dry powder inhaler; DPPC = dipalmitoylphosphatidylcholine; EC = ethyl cellulose; EGG = excipient enhanced growth; FDA = Food and Drug Administration; FDKP = fumaryl diketopiperazine; HA = hyaluronic acid; MDI = pressured metered dose inhaler; NaAlg = sodium alginate; NaCit = sodium citrate; NaCMC = sodium carboxymethylcellulose; NEB = nebuliser; PA = Pseudomonas aeruginosa; PEG = polyethylene glycol; PLGA = poly(lactide-co-glycolide); PS = polysorbate; PVP = polyvinylpyrrolidone; PX = poloxamer; SD = spray-dried; SorbMO = sorbitan monooleate.

a FDA values stated in parentheses are the maximum daily exposure or the maximum potency per unit dose listed in the public FDA inactive ingredient database at the time of writing for similar preparations for inhalation and are intended for reference only. Other values may apply depending on individual circumstances (https://www.accessdata.fda.gov/scripts/cder/iig/index.Cfm).

b NA = not applicable, since the compound was a principal component of the carrier particle in the study, absolute amount of the compound per dose was dependent on the loading efficiency, which was a variable in the cited studies.

c NA = not applicable, since the process enhancer decomposed and was not intended to be present in the final formulation.

providers are not able to demonstrate the correct pMDI technique.86–89

DPIs offer another option for inhaled therapies with a number of advantages including ease of use, convenient portability and solid-state stability compared to liquid formulations.82,83 In contrast to the use of pMDIs, the breath-activated dispersion mechanism of most DPIs means that coordination between inspiration and actuation is not required. In addition to the conventional multi-dose therapies typical for airway conditions, they can also be available as simple disposable devices for single-dose treatments such as vaccines.10 However, DPIs cannot be used with spacers which may be a consideration for patients who have to inhale large doses of drugs.84 DPIs have also long been deemed unsuitable as acute reliever for asthma, for which pMDIs were typically chosen for the rapid onset of action required. Recent studies have shown that the budesonide/formoterol combination DPI can be an effective option as both maintenance and reliever medicine in asthma.85–87 This may potentially inspire new developments of DPIs in this space.

**Formation and Excipients**

The formulation considerations and challenges vary depending on the choice of delivery system. The selection of formulation and excipients depends on several factors including the properties of the API, the mode of delivery and the safety profiles of the excipients in lungs. While a variety of excipients have been studied in the
literature, only a limited number of excipients have been approved for pulmonary use (Table 3). It is perhaps not surprising considering that regulatory agencies tend to favour the use of commercially established excipients as well as generally recognised as safe (GRAS) substances. In this regard, commercial products, and therefore excipients, approved for inhalation are relatively few compared to orally administered agents. In general, an excipient is assumed to be approved when a new drug formulation containing the excipient receives regulatory acceptance. However, published literature and regulatory guidance on the assessment of excipients for inhalation is still very limited, with a lack of concerted international guidelines directly relating to the safety evaluation of pharmaceutical excipients.11,88

Different aerosol delivery systems require different excipients in their formulations. Liquid formulations for NEBs should be adjusted to physiological pH and osmolarity to avoid the potential induction of cough and bronchoconstriction.89–91 Acidic and basic saline solutions of pH <4.5 and >8.7, respectively, have been shown to induce apnoea in puppies.92 Unlike the GI tract, the buffering capacity of lungs is limited. For pH adjustment, hydrochloric acid, sodium hydroxide, citric acid and phosphates are commonly used. For osmolarity, sodium chloride is most widely used in these formulations. Low amounts of surfactants such as polysorbates have been used to improve solubility of drugs as well as dissolution and dispersion of particles in suspensions. Co-solvents such as ethanol may only be used in limited amounts to minimise potential irritation to the lungs11 (Table 3).

Similar excipients as those used in NEBs are also used in pMDI formulations. However, compatibility of the propellant system, which comprises the bulk of pMDI formulations, also needs to be considered. The most important change in pMDI formulations occurred when chlorofluorocarbon propellants (CFC) was found to be involved in the depletion of stratospheric ozone.93 In response to this finding the Montreal Protocol was then devised triggering the transition of CFC to the more environmentally friendly hydrofluoroalkane propellants (HFA).94 However, the physical and solvency properties of HFAs are very different to those of CFCs. Many drugs and excipients that are soluble in CFCs are not readily soluble in HFAs.95 Suggested strategies in reformulation include the addition of co-solvents, the development of new surfactants, and particle engineering to produce more HFA-compatible materials.11 While HFAs have no potential to deplete the ozone layer, it should be noted that HFAs are powerful greenhouse gases. To this end, the breath-activated DPIs are more environment friendly as they remove the need of propellants entirely. Strategies to switch pMDIs to DPIs with lower global warming potentials are actively being explored.96–98

The solid nature of DPI formulations means that formulation performance is largely dependent on the physical properties of the particles (e.g. particle size, flow properties, surface energy, porosity, dispersibility and crystallinity) and particle engineering is therefore of critical importance. The more conventional top-down particle manufacturing methods (e.g. various milling and micronisation techniques) obtain small particles by physically breaking down large particles. These methods, albeit their ability to provide small particles, provide little control over other properties of the resultant particles. The bottom-up particle manufacturing methods (e.g. spray-drying, spray-freeze-drying and supercritical fluids) manipulate particle formation from the molecular level and provide more opportunities for particle engineering. Given sufficient understanding of particle formation mechanisms, particles can be carefully engineered to provide multiple functionalities for improved stability and aerosolisation. The complications in designing fine particles for pulmonary drug delivery have been thoroughly discussed in the literature.82,99

With the increasing interest in inhaled drug delivery, the development of new and improved excipients with different functionalities for inhalation has expanded in recent years (Table 3). In addition to improving dispersibility and aerosolisation, excipients are also being used to ameliorate other formulation properties. For instance, a number of amino acids, small carbohydrates and polymers have been used as glass-formers or stabilisers for stabilisation of biopharmaceuticals.100–102 Excipients have also been used as mucoadhesive agents to prolong residence time and release of drugs delivered to the respiratory tract. For example, chitosan has been used to facilitate particle adherence to cell membranes.103 Poly(lactide-co-glycolide) (PLGA) has been used to provide a carrier matrix to achieve a sustained-release profile of the API.103,104 While these excipients might also be used in other routes of administration for similar purposes, given the complexity in engineering aerosolisable particles, incorporating them into formulation suitable for inhalation is another art.

**Device**

The design of a device can influence the delivery efficiency of the formulation and in turn the efficacy of the product. The delivery efficiency of liquid formulations for nebulisation, for instance, is largely dependent on the performance of the nebuliser. Common types of NEBs include jet nebulisers, vibrating mesh nebulisers and ultrasonic nebulisers. The use of jet nebulisers and vibrating mesh nebulisers has been reported since as early as the 1950s.72 These nebulisers, despite the long history and extensive experience, are generally inefficient and highly variable for drug delivery. Depending on the design of the nebuliser and the interface used (e.g. mouthpiece, aerosol mask and valved-mask), the delivery efficiency has been reported to range from 7% to 35% in adults and could be as low as 4% in paediatrics, whereas dose retention in the device could be as high as 75% with the majority of the formulation deposited in the nebuliser itself.105,106 Ultrasonic nebulisers, albeit being more efficient than jet nebulisers, have large residual volume and are not suitable in aerosolising viscous formulations.107,108 Interested readers are referred to the literature for information about the range of factors impacting on nebulisation performance and detailed comparisons of various nebulisers.109,110

Liquid formulations can also be delivered by pMDIs, for which key components including the propellant, the container, the actuator and the metering valve, in addition to the drug formulation, are essential.112,113 The canister must be able to withstand the high pressure generated by the propellant and inert so to not adversely interact with the formulation. The coating on the internal surface of the containers have been used to prevent interactions with formulations. Typical materials for internal coatings on the surface of aluminium canisters include epoxy resins, anodized aluminium, epoxy-phenol or perfluoroalkoxy alkane.114 The valve should be made of materials compatible with the propellant, the excipients and the solvents in the formulation. For instance, during the transition of CFC to HFA, compatibility of metering valve elastomers with HFA formulations had to be evaluated to ensure proper functioning of the valves.105,106 Both solution and suspension formulations can be delivered by pMDIs when formulated appropriately.67 Since delivery efficiency of pMDIs is highly dependent on patient coordination and inhaler technique, which can be challenging for some populations such as children or elderly, breath-actuated pMDIs that fire the dose automatically upon the patient’s inhalation have emerged on the market. The actuation of Autohaler®, for instance, is triggered by a vane mechanism and has been shown to improve drug deposition in patients who are poor coordinators.117 For more sophisticated design, SmartMist®...
contains a microprocessor and actuates only when a pre-
programmed combination of flow and volume is achieved.118

DPIs available on the market are primarily passive dispersion
device in which the powder formulation is aerosolised upon
inspiration by the patient.119 These devices are therefore by nature
breath-actuated without the necessity of patient coordination.
Powder dispersion and entrainment is a complex phenomenon
governed by a number of mechanisms such as drag force, particle-
particle collision, agglomerate–device impaction and turbulence
generated upon inspiration.120–122 The design of a device plays a
crucial role in these mechanisms and thus dispersion perfor-
mance.124,125 Recently, active dispersion devices, which actively
disperse the powder formulation with an energy source to produce
an aerosol before inspiration, have also started to emerge. Since the
powder aerosol is generated by the device instead of the patient’s
inspiratory effort, these devices enable improved dosing precision
with reproducible aerosol production independent of respiratory
force. The generated aerosol cloud is retained in a chamber to be
inhaled by the patient. The first approved inhaled insulin delivery
product, Exubera®, is a notable example of an active DPI device
which aerosolises the formulation by compressed air generated by
the patient.120 The product was later withdrawn after failing to gain
market acceptance from physicians and patients due to factors
including lack of marketing, perceived indiscretion and the associ-
ated high cost compared to conventional insulin products.127

Nevertheless, the development programme was a massive tech-
nical achievement and demonstrated the benefits of active devices
and the feasibility of systemic delivery of biopharmaceuticals via
pulmonary administration using DPIs. The role of devices and the
wide range of designs have been discussed in the literature.128

The Intricate Relationship between the Mode of Delivery,
Formulation and Device

It is important to appreciate the inseparable and intricate rela-
tionship between the mode of delivery, formulation and device. The
delivery efficiency and particle deposition pattern in lungs could
differ substantially when a formulation is delivered in a different
setting. For instance, the effectiveness of tobramycin differs
dependent on the choice of delivery system with reported differ-
ences in relative bioavailability being as much as nine times when
delivered as a carefully engineered dry powder formulation
compared to nebulisation.28,130 The capsule device configuration
has been shown to have an effect on the energy input for powder
dispersion, and consequently fine particle fractions, powder
emptying and impaction loss.131 In another study, the emitted
fraction of a dry powder formulation varied between 58 and 87%
when tested in different combinations of capsules and devices.132

The addition of fines in the carrier, as another example, has been
reported to be beneficial in improving formulation perform-
ance.133–134 The effect of fines, however, became less relevant in a
device employing inertial separation as the dispersion mechanism,
which is by nature more effective than other separation forces such
as drag and lift, and the formulation may perform even better
without large quantities of fines.135–137

The contrasting behaviours of the same formulation in different
delivery settings highlight the important interactions between the
components of the trio. The performance of a formulation cannot be
directly translated to all devices and delivery settings. The selec-
tion of excipients depends on many factors including, but not
limited to, the API properties, the dose required, the process con-
ditions and the device. Therefore, the formulation and the device
have to be co-developed for the intended mode of administration
for optimal results. It is crucial to consider details of every
component in the trio and the interactions between them carefully
during the development process to define an optimal pulmonary
drug delivery product.

Animal Models in the Development of Pulmonary
Formulations

Small Animal Models

Small rodents such as mice, rats and guinea pigs have been used
as the animal model of choice in virtually all initial studies of
pharmaceutical research. Compared to large animals, the lower cost
in housing and handling and the relative ease of terminal proced-
ures allow the use of a higher number of animals for statistical
validity. Although there are differences in pulmonary physiology
compared to human, these small animals have been instrumental
in pilot studies to demonstrate the feasibility of delivering a com-
pound via the pulmonary system. Mice, for instance, have been
used as a model to study pulmonary delivery of genes, antifungal
and anticancer agents in a variety of conditions including cystic
fibrosis (CF), lung cancer and COPD.101,138–140 Rats have been used
to study the effect of bronchodilators, antimicrobials and vaccines
after pulmonary administration.140–146 Guinea pigs have been the
most commonly used small animal model for asthma and COPD
due to the many similarities in pulmonary physiology compared
to humans including airway control and response to allergens.147,148 It
has also been used as a model for pulmonary infectious diseases
such as tuberculosis to study the PK of its treatment after pulmo-
nary administration.149

Large Animal Models

The lung anatomy and the respiratory physiology of large ani-
mals such as rabbits, sheep, dogs and macaques are much different
from that of small rodents. The larger lungs of these animals pro-
vide a more representative environment for particle transfer and
deposition in lungs compared to smaller animals. For example,
intubated rabbits have been used to study the PK of a dry powder
aerosol of vancomycin after pulmonary delivery.150 Although
endotracheal tubes were used to bypass the mouth and throat, the
study demonstrated the feasibility of locally delivered antibiotics
for the treatment of pulmonary infections, revealing the potential
for improved efficacy and reduced systemic exposure using local
lung delivery. Rabbites have also been used to study the systemic
absorption and exposure of nebulised insulin via pulmonary
administration.151,152 Further, dogs have been used as animal
models in a number of studies. For examples, conscious Labrador
dogs have been used for safety evaluation of an aerosolised car-
 diovascular drug administered using a novel inhaler device.153
Beagle dogs have been in used in a number of studies as a model to
study systemic exposure of aerosolised dry powder formulations
of insulin.154–156 Sheep have been suggested as a suitable animal
model to study treatments of respiratory conditions such as
asthma, COPD and CF.157–160 In addition to the similar pulmonary
physiology compared to humans, the model allows repeated sam-
pling of airway cells and tissues as well as measures of airway
functions that are not possible in small animal models.

Non-human primates such as monkeys have been assessed as an
animal model for COPD and asthma. The structural components
of their airways – e.g. smooth muscles, cartilage and submucosal
glands – are more similar to humans than other animal models.159
Monkeys have been used to study local alveolar deposition and
delivery of antibodies after aerosolised delivery into lungs.160 Ma-
caque have been used to study measles vaccination via inhalation
of a dry powder aerosol vaccine.161 These animals are likely to be
reserved for studies in the later stages of development. With the more relevant size and branching structure of the airways compared to humans, these animal models can be invaluable in studying drug delivery efficiency and efficacy as a function of regional particle deposition pattern within the respiratory tract and aerosolisation performance, which might not be possible in smaller animals. The pulmonary physiology and respiratory parameters of different animals are summarised in Table 4.

Selection of Animal Models

The choice of animal model is dependent on the research question of interest. While small rodents are more commonly used in research, the respiratory physiology of larger species such as sheep and monkeys provides a more relevant environment for complex study designs and extrapolation to humans. The values of small rodents in early-phase pharmaceutical studies are widely recognised. These small animal models can provide valuable insights into the feasibility of delivering a therapeutic agent via the pulmonary system. The in vivo efficacy and local toxicity of potential drug candidates can be studied in these small animal models to examine their therapeutic potential following pulmonary administration. With the increasing interest in systemic delivery of therapeutic agents via the respiratory tract, these animals can also provide an indication on the systemic exposure of drugs after pulmonary delivery.

Drug particles deposited in different regions of the respiratory tract face different fates and, subsequently, induce different therapeutic actions. These properties cannot be easily and reliably assessed in small rodents given the small size of their lungs and differences in pulmonary physiology. In contrast, larger animals have been used as disease models of respiratory infections of Pseudomonas aeruginosa and these models may be used to study the effects of antimicrobial agents. Given the similar symptoms and immune responses compared to humans, guinea pigs have been used to study various infections including tuberculosis and diphtheria. Sheep have been suggested to be a suitable model for the study of a wide range of respiratory diseases including asthma, chronic bronchitis, emphysema and CF. Ferrets have long been used as a model for the study of influenza infections. Depending on the disease of interest, these animals can be useful models to study different pulmonary therapies.

In Vitro-In Vivo Correlation

In vitro testings and performance assessments have been largely focused on the aerosolisation properties of formulations. The aerosolisation performance of a formulation is commonly measured by its aerodynamic particle size distribution (APSD), typically evaluated using cascade impactors or liquid impingers (Fig. 3). These instruments operate on the principle of inertial impaction and are listed in the U.S. and European Pharmacopoeias for aerodynamic assessment. Small particles with low densities are aerodynamically more favourable than larger and heavier particles, and are thus able to travel further to the later stages of the impinger or the impactor. The simplest instrument for APSD measurement is likely the glass Twin-Stage Impinger (TSI), in which particles are separated into two groups depending on their aerodynamic diameters. The Multi-Stage Liquid Impinger (MSLI) operates on the same principle as the TSI but can separate particles into four stages. Particles with aerodynamic diameters larger than the stage cut-off diameter are trapped in the liquid at that stage.

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The particle collection mechanism in cascade impactors does not require liquid in the stages. Particles are impacted directly at the surface of each stage. Cascade impactors including the Andersen Cascade Impactor (ACI) and the Next Generation Impactor (NGI) have been commonly used in various studies. In recent years, the concept of abbreviated impactor measurement (AIM) has been introduced as a simpler and faster assessment tool compared to full-resolution impactor testing. Depending on the stage in the development process, a full-resolution APSD characterisation may not be needed. For instance, AIMs can be used as a screening method in early stage development, as well as a quality assurance method once the product is fully characterised. AIMs such as Fast Screening Andersen (FSA) and Reduced Next Generation Impactor (RNGI), an abbreviated set-up of the ACI and NGI, respectively, have been introduced. Compared to full-resolution impactor testing, the abbreviated settings provide a more efficient APSD measurement methodology allowing higher throughput screening of formulations. These AIMs have been evaluated in recent studies and

Table 4

Typical Respiratory Physiology Parameters of Different Animal Models in the Literature.

| Parameters                        | Human | Monkey (Rhesus) | Sheep | Dog | Rabbit | Guinea Pig | Rat | Mouse |
|-----------------------------------|-------|-----------------|-------|-----|--------|------------|-----|-------|
| Size (kg)                         | 70–80 | 2.0–6.7         | 40–82 | 10–16| 2.5–4.5| 0.4–1.0    | 0.25–0.35 | 0.02–0.04 |
| Lung symmetry                      | Dichotomous | Monopodial | Dichotomous | Monopodial | Monopodial | Monopodial | Monopodial | Monopodial |
| Lung weight (g)                   | 1000  | 33              | 388–810| 100 | 18     | NA         | 1.5  | 0.12  |
| Lung volume (mL)                  | 4341  | 143             | 2162–4418| 736–1322| 79.2    | 13         | 8.6  | 0.74  |
| Surface area of the alveolar region (m²) | 143  | NA              | NA    | 40.7 | 5.8    | NA         | 0.4  | 0.07  |
| Diameter of alveoli (μm)          | 219   | NA              | NA    | 126 | 88     | 65         | 70   | 47    |
| Alveoli number (< 10⁶)             | 950   | NA              | NA    | 1040| 135    | 69         | 43   | 18    |
| Alveolar macrophages (< 10⁹)      | 5990  | NA              | NA    | 3940| 142    | 58.8       | 29.1 | 2.9   |
| Lining fluid volume (mL)          | 20–40 | NA              | 11.4  | 167 | 1.22   | NA         | 0.045–0.055 | 0.005–0.015 |
| Nose/mouth breather               | Nose/mouth | Nose/mouth | Nose/mouth | Nose/mouth | Nose     | Nose       | Nose  | Nose  |
| Respiratory rate (min⁻¹)          | 15    | 39              | 40    | 23  | 51     | 90         | 85   | 163   |
| Tidal volume (mL)                 | 400–616| 21              | 203   | 11.4–16.6 | 15.8    | 1.72–1.75  | 0.87–2.08 | 0.15–0.18 |
| Mucous clearance (mm/min)         | 3.6–21.5| NA              | 7.4–11.9| 7.5–21.6| 3.2     | 2.7        | 1.9–5.9 | NA    |
| Particle range for alveolar deposition (μm) | 1–5  | 1–5             | NA    | 1–3 | NA     | NA         | 3.5  | 3     |

Abbreviations: NA = not available.

* Values are typical values reported in the literature and may vary depending on the size of the animal.
were shown to be in good agreement with full-resolution impactors in a number of metrics including coarse mass, fine particle and extra-fine fractions.\textsuperscript{170,171} Ideally, it would be preferable to evaluate in vitro aerosolisation at a range of conditions (e.g. flow rate, temperature and humidity) so to take into consideration the overall performance of the formulation under different ambient settings and disease status.

As described in Section \textit{Physiology of the Respiratory System} above, particles deposited in different regions of the lungs encounter vastly different physiological barriers. For the study of drug interactions with lungs and absorption across airway epithelia, a number of in vitro models of airway epithelia have been used. Cell lines of human bronchial epithelial origin such as Calu-3, 16HBE14o- and BEAS-2B and alveolar origin such as A549 have been explored for their use in pulmonary absorption studies. Calu-3 and 16HBE14o- both form tight junctions and have been suggested to be good in vitro models for drug absorption and transport.\textsuperscript{172–174} However, Calu-3 also produces large quantities of secretory components consistent with the properties of the serous cells of the tracheobronchial gland.\textsuperscript{175} Calu-3 has therefore been used in numerous studies for a wide range of applications such as pulmonary drug absorption, transport and metabolism.\textsuperscript{176–178} Another commonly used cell-line is BEAS-2B, which has been widely used for the study of pathological processes and drug metabolism. However, BEAS-2B does not form tight junction as readily as Calu-3 and 16HBE14o- and thus its use as a model of drug transport has been limited.\textsuperscript{175} The suitability of A549 as a model has also been debated since it is functionally deficient in tight junctions and its ability to detect chemically induced alveolar toxicity has been questioned.\textsuperscript{179} With the advancement in cell culture techniques, 3D tissue models of human airways produced from primary healthy and diseased human cells such as EpiAirway\textsuperscript{TM} from MatTek can now also be found on the market for drug delivery applications. The potential impact of disease status on drug absorption may also be investigated by simulating different airway conditions (e.g. permeability and mucus production).\textsuperscript{180,181} Recently, the use of an impactor-integrated cell culture model, combining NGI with a culture of human alveolar A549 epithelial cells, to study the dissolution and uptake of drugs following lung deposition has also been reported.\textsuperscript{182}

For the study of mechanisms governing drug transport or lung disposition that cannot be readily examined with in vitro or in vivo models, ex vivo models can be useful options. The most common and useful ex vivo model is the isolated perfused lung (IPL) from rats, where the lungs are isolated and the pulmonary circulation is perfused through with blood or a buffered solution at pH 7.4. The use of the model in pharmacological studies for endogenous and exogenous substances as well as its application in the study of pulmonary uptake and metabolisms of drugs are well-documented.\textsuperscript{183–186} Since its introduction, the model has been adapted for the study of drug absorption from the airways.\textsuperscript{187–190} The principles and different experimental setups of the IPL and the various applications of the model have been recently discussed in the literature.\textsuperscript{191–195} The ex vivo model eliminates the confounding factors from whole body complications in in vivo studies, allowing control of perfusion with multiple and frequent sampling. However, it is worth noting that the IPL model does not include the bronchial circulation serving the upper airways including the bronchi, bronchioles and trachea. Hence, the model may largely underestimate the contribution from the tracheobronchial airways on drug...
absorption. The impact will likely be specific to the drug and formulation and will need to be evaluated on a case-by-case basis.

**In Vivo Assessments and Clinical Studies**

In vivo studies of inhaled pharmaceuticals performed in animals are largely used as pilot studies to investigate efficacy of an agent after pulmonary administration. These studies are performed to verify the drug exposure, efficacy or toxicity of an optimised formulation, rather than comparing formulation properties that can be assessed in vitro. For instance, an optimised formulation of chitosan-coated PLGA nanoparticles had been first tested in vitro for its release profile and intracellular uptake before being administered into the lungs of rats to show its low irritability to lungs in vivo. The PK and glycaemic control of two insulin dry powder formulations have been studied in dogs after pulmonary administration. The thermal and hygroscopic properties of these formulations were initially tested in vitro before being shown to have comparable in vivo efficacy in dogs. Ciprofloxacin liposomes, 1,25-dihydroxyvitamin D3 and dry powder vaccine formulations for influenza and measles have been tested in mice, rats and macaques to study their efficacy following pulmonary administration. These studies were intended to verify the feasibility of delivering the agents or formulations via the pulmonary route rather than comparing the in vivo efficacy of different pulmonary formulations.

Other studies used animal models to study in vivo performance resulting from the differences in formulation properties and processes. One study compared the PK properties of microstructured crystalline and nanostructured amorphous voriconazole formulations in mice after pulmonary administration. While the amorphous formulation demonstrated a faster release rate, its PK was less favourable with a lower area under the curve (AUC) from the plasma concentrations vs. time profiles in lungs and plasma. The faster release and absorption of the amorphous formulation resulted in more rapid elimination of the drug from plasma, while the microstructured crystalline formulation acted as a reservoir of drug in lung tissues. In another study, a tobramycin dry powder formulation produced a higher AUC compared to a nebulised formulation. The regional deposition pattern of particles within the respiratory tract, as discussed above, is a crucial determinant of the FPF, the lung dose, the systemic absorption and the therapeutic efficiency in pulmonary formulations. In recent years, in vivo imaging techniques such as gamma scintigraphy, single photon emission computed tomography (SPECT) and positron emission tomography (PET) have been used to study deposition patterns of inhaled aerosols. While routine in vivo imaging and measurements of regional disposition patterns of all formulations could be logistically challenging, in vitro particle aerosolisation measurements are regularly performed to characterise inhaled formulations. The relationship between in vitro particle aerosolisation and in vivo deposition pattern of inhaled formulations may hence provide an avenue to the establishment of IVIVC for these formulations. The in vitro aerosolisation performance of an inhaled formulation is typically evaluated by characterising its APSD using cascade impactors. While providing a common standard for product specifications and quality control purposes, APSD results from impactor studies do not necessarily reflect in vivo particle deposition accurately and the relationship between APSD and clinical response remains to be elucidated.

**Challenges in Establishing IVIVC**

There have been attempts to understand the structure-activity relationship of compounds for pulmonary absorption. The subsequent development of an inhalable formulation for the optimised drug candidate, however, is not straightforward. Given the high permeability of the respiratory zone, the pulmonary route can be highly effective for systemic drug delivery of biomacromolecules that cannot be easily absorbed via the GI tract such as insulin, antibodies and vaccine antigens. However, the absorption of inhaled formulations from the lungs is complex. The use of standard in vitro dissolution and solubility tests as predictors of systemic absorption for oral formulations is not directly applicable for inhaled formulations. The typical dissolution tests developed for oral formulations are not valid for pulmonary delivery due to the differences in pH and typical volume of fluid available for dissolution. To this end, there has been ongoing efforts in the development of physiologically relevant simulated human lung fluids and dissolution techniques for inhaled drugs.

It is worth noting that, a well-designed dry powder formulation can provide better bioavailability compared to a solution formulation. In one study, due to the more favourable deposition profile and superior delivery efficiency, a dry powder formulation was able to improve relative bioavailability by 9-fold compared to a solution formulation. Furthermore, depending on the clinical indication, e.g. asthma and COPD, local drug retention within the airways may be more desirable than systemic absorption. Strategies such as reducing dissolution rate of the aerosolised particles and lowering permeability of the compound may then be employed to improve lung retention with reduced systemic exposure. Hence, the comparison of systemic bioavailability per se is unlikely to be a universally relevant parameter for the assessment of these formulations. It is clear that the IVIVC of inhaled formulations remains to be established.

The regional deposition pattern of particles within the respiratory tract, as discussed above, is a crucial determinant of the FPF; the lung dose, the systemic absorption and the therapeutic efficacy of inhaled formulations. In recent years, in vivo imaging techniques such as gamma scintigraphy, single photon emission computed tomography (SPECT) and positron emission tomography (PET) have been used to study deposition patterns of inhaled aerosols. While routine in vivo imaging and measurements of regional disposition patterns of all formulations could be logistically challenging, in vitro particle aerosolisation measurements are regularly performed to characterise inhaled formulations. The relationship between in vitro particle aerosolisation and in vivo deposition pattern of inhaled formulations may hence provide an avenue to the establishment of IVIVC for these formulations. The in vitro aerosolisation performance of an inhaled formulation is typically evaluated by characterising its APSD using cascade impactors. While providing a common standard for product specifications and quality control purposes, APSD results from impactor studies do not necessarily reflect in vivo particle deposition accurately and the relationship between APSD and clinical response remains to be elucidated.

To this end, the United States Pharmacopeia (USP) induction port (IP) is commonly used in impactor studies to provide a common standard for the collection of particles likely to deposit in the oropharynx region. Although commonly referred to as the USP throat in publications, it is important to emphasise that there are major differences in geometry between the rather simple USP IP and a human throat. Studies have been conducted using the mouth-throat region of the human cast as well as an idealised mouth-throat replica to study particle deposition in the oropharynx region. These studies showed that the USP IP is not a good model for...
simulation of mouth–throat deposition. Hence, compared to impactor studies, the use of more anatomically correct models of the respiratory tract for the study of regional deposition would be valuable. In addition, it has been discussed in the literature that the relationship between APSD and clinical response is not always apparent. Depending on the type of drugs, APSD can vary considerably with little change in clinical response. It is striking that only a few studies have been designed to specifically study the relationship between APSD and clinical response. While there is little doubt that APSDs affect particle deposition patterns and, subsequently, clinical responses, the relationship between them is not straightforward. In the absence of information on lung deposition pattern, APSD per se, while offering an indication to clinical efficacy, does not accurately predict it.

Bioequivalence

The challenges in establishing IVIVC has direct implications on bioequivalence and bioavailability studies. Inhaled drug products are complex since the performance of the product is dependent on the interplay of the mode of delivery, formulation and device as discussed above. Hence, in addition to the composition of the formulation, the design of the device and its interaction with the formulation also needs to be considered. Consequently, demonstrating bioequivalence of inhaled drug products is not straightforward, as indicated by the lack of harmonisation between regulatory guidelines in different regions. The FDA uses an “aggregate weight-of-evidence” approach that considers in vitro studies, PK studies, and PD or comparative clinical endpoint studies, along with the potential impact from a test product’s formulation and device design on bioequivalence. In contrast, the EU adopts a step-wise approach under which bioequivalence is apparent. Depending on the type of drugs, APSD can vary, relationship between APSD and clinical response is not always valuable. In addition, it has been discussed in the literature that the respiratory tract for the study of regional deposition would be

To this end, the key considerations in the in vitro testing of inhaled drug products to support a science-based regulatory approach for the approval of inhaled pharmaceuticals have been summarised in the literature. In addition to biopharmaceutical properties, other factors such as patient demographics, inhaler use technique, lung disease status and choice of appropriate statistical models for data analysis and hypothesis testing were also discussed by the authors. Recently, the use of exhaled breath condensate (EBC) samples as an alternative method to demonstrate bioequivalence of inhaled drug products has been proposed. EBC analysis can measure drug concentrations in airway lining fluids directly allowing comparison of local PK in an efficient and non-invasive manner. Future studies are required to evaluate the applicability of this novel approach in bioequivalence studies.

Model-Informed Formulation Development for Inhaled Pharmaceuticals

Molecular Dynamics Simulations for Particle Engineering

Molecular dynamics (MD) uses Newton’s equations of motion to computationally simulate the time evolution of a set of interacting atoms and molecules. MD has traditionally been used as an in silico tool to support drug discovery and design. It can help the study of drug binding and provide insights into ligand-receptor interactions. In recent years, MD simulations have also been applied to study molecular interactions of components in dry delivery systems. For pulmonary drug delivery, it has been used to investigate the complexion of celecoxib and cyclodextrin intended for a dry power formulation and the potential effect of glycerol on the bioavailability of inhaled steroids by simulating the interactions of glycerol with model pulmonary interfaces. In another example, coarse-grained MD was performed to investigate whether PEG encapsulation could be used to enhance pulmonary absorption and permeation of an antimicrobial peptide. Another study used MD for the prediction of carrier behaviour in a polar in vivo condition. These studies used MD to simulate the interactions of excipients and drugs to investigate their potential fate following lung delivery. Moreover, MD simulations have also been used to study the structure of multicomponent aerosol nanoparticles under atmospheric conditions as well as particle formation in a supercritical solution. Hence, with the appropriate setup, MD may potentially be applied to study particle formation mechanisms to advance particle engineering for inhaled formulations.

Particle Deposition Modelling for Lung Distribution Pattern

It can be conceptualised that there are three primary determinants dictating particle deposition patterns in the airways: formulation properties, respiratory anatomy and breathing parameters. Formulation and particle properties such as APSD, electrostatic charges and morphology can be measured and controlled in an in vitro setting. Respiratory anatomy, which is dependent on the species of interest and its disease status, can be investigated independent of the formulation. The specific information about the airways of the species and their conditions at different disease status can then be incorporated accordingly. Breathing parameters, on the other hand, dictate the energy and fluid dynamics concerning the travelling of particles with a given set of properties, e.g. APSD, through the bronchialveolar tree of the species. These factors and their interplay need to be taken into account appropriately to predict particle deposition pattern.

Particle deposition models within the respiratory tract could help bridge the gap between APSD and in vivo response for inhaled
Therapies. In silico models such as computational fluid dynamics (CFD) with realistic anatomies and complex flow fields have been used to study inhaled particle deposition.\textsuperscript{225–227} A well-validated multiple path particle dosimetry (MPPD) model, combining information from CFD and lung anatomical models, with APSD and breathing parameters as input variables, might bring us closer towards establishing the IVIVC of inhaled formulations.\textsuperscript{228} The modelling of inhaled particle deposition in the human lungs has been reviewed in detail in the literature.\textsuperscript{229} By incorporating the airflow conditions at different disease status (e.g. airway narrowing, mucus production and reduced flow rate), the potential impact of the disease on deposition pattern may also be investigated.

A recent study reported the use of artificial neural network (ANN) to predict FPF by incorporating the effect of API properties, formulation factors and device factors.\textsuperscript{330} Although larger datasets and more input variables are needed to realise its full potential, the study has demonstrated the feasibility of this approach and the importance of these factors on the respirability of particles. Machine learning approaches are typically heavily data driven. In general, the algorithms of choice search for associations between the available set of variables and the outcome of interest in the dataset in order to develop predictive models. While these models focus on maximising predictive performance, there is often little mechanistic insight or learning in the model, which could be a black box impossible to dissect for detailed information. The interpretation of the influential variables therefore relies on the investigator’s understanding of the system.

Translational Modelling from Preclinical Species to Human

In recent years, efforts to quantitatively study the PK of inhaled drugs using mechanistic computational models such as multi-compartment physiologically-based pharmacokinetic (PBPK) and systems pharmacology models have gained increased interest.\textsuperscript{231–236} By building a computational lung model using a bottom-up approach, capable of describing the absorption and the distribution of drugs following lung administration, one can simulate scenarios to investigate the effect of different branching structures and respiratory anatomy for different species and disease conditions. By combining a particle deposition model and a human PK model with lung absorption, it is then possible to project human PK from APSD information during formulation characterisation\textsuperscript{317,238} and hence facilitate formulation design. Some commercial packages for PBPK modelling now have modules available for lung administration. Compared to the extensive experience of PBPK models for oral administration, the usability and reliability of these packages for lung administration remains to be validated. These physiologically-based modelling approaches provide a means to predict potential drug absorption and distribution for preliminary evaluation before in vivo data are available.

When PK data become available on the compound of interest from in vivo studies, modelling approaches using the PK data should be considered. Good analysis and modelling require scientifically sound understanding and interpretation of the data. In contrast to systemic plasma PK, the investigation of lung PK presents its unique challenges. For the study of local PK in lungs, different sampling methods have been used to measure lung concentrations — e.g. sputum samples, bronchoalveolar lavage fluid (BALF), microdialysis and homogenised lung tissues — and it is important to note that these methods do not necessarily provide the same information. While drug concentrations in sputum and BALF samples are more representative of drug concentrations in the epithelium lining fluid (ELF), microdialysis provides information on free drug concentrations in the interstitial fluid at the site of interest. In contrast, drug concentrations in lung tissue homogenates are not informative on whether the amount of drug is available for activity. However, it can be useful in initial studies to determine the overall distribution of the drug during the early phase of development.\textsuperscript{239} These sampling methods might be applicable for different treatments depending on the target site of action. For instance, ELF concentrations may be more relevant for an extra-cellular target (e.g. extracellular pathogen of a lung infection), while interstitial concentrations may be more applicable for airway conditions (e.g. asthma and COPD). The analysis of these PK data should therefore take into account the nature of the sampling method. The modelling of ELF concentrations using BALF data is relatively common in the literature with some studies using lung tissue homogenates in the early phase of development.\textsuperscript{168,240–243}

In addition to PK, developing pharmacodynamic (PD) models to describe drug effect following pulmonary administration is another challenge. PD models differ depending on the disease of interest. The development of models for the translation of drug effect from preclinical species to humans requires understanding of the disease mechanisms and the exposure-response relationship. Translational PKPD models can then be built to integrate all preclinical data and information available in a mechanistic manner to support prediction of human doses for efficacy. These model-based approaches are not unique to pulmonary delivery and have been advocated in other areas for clinical translation and dose prediction.\textsuperscript{244–246} Efforts to more specifically apply these modelling approaches in the development of inhaled pharmaceuticals have started to emerge.\textsuperscript{247–249} The increasing application of these computational methods can potentially improve development efficiency, reduce the use of animals in preclinical studies and help understand processes of importance to achieve, for example, high local concentration or systemic exposure.

Pharmacometrics and Biopharmaceutical Modelling

In contrast to systems pharmacology-based models that are developed to quantitatively describe a biological or disease process with less emphasis on describing specific observations, if they are available at all, pharmacometric models are developed based on the available data. These models are typically developed relying on robust statistical models or algorithms derived to describe the data, and are rigorously assessed for their ability to reproduce the observations.\textsuperscript{250} During the early research and discovery phases of new drug candidates, scientists start with no in vivo data on the compounds and preparation of first-time-in-human (FTIH) trials is the key. In the absences of any observations (e.g. in vivo drug concentration and effect data), bottom-up system-based modelling approaches (e.g. PBPK) can be applied to predict potential outcome given the understanding of the system (e.g. biology of the animal species) and the in vitro measurements of the drug (e.g. solubility, permeability a metabolism). As more preclinical and clinical data become available, it can then be appropriate to use the more data-driven modelling approaches (e.g. pharmacometric and non-linear mixed effects (NLME) modelling) to relate drug input directly to the observations in order to identify influential parameters and improve predictive capacity. Depending on the study design and the richness of the data, interindividual variability and covariate identification at the population level can also be included in the modelling and analysis. Therefore, these different modelling approaches are complementary to each other and can be applicable at different stages of the drug development process (Fig. 4). The potential roles of some of these modelling approaches in the development of orally inhaled drugs have been reviewed in the literature.\textsuperscript{212}

In a similar vein, as more in vivo data from different formulations become available, it is then also possible to develop models to
**Fig. 4.** Examples of modelling and simulation approaches with their key attributes and potential applications at different stages of the drug development process in modern development of inhaled pharmaceuticals.

Recent reports on the applications of various computational techniques in the development of inhaled pharmaceuticals have demonstrated the utilities of these in silico approaches. MD may be used to study particle formation mechanisms to advance particle design and engineering. For instance, pharmacometric models incorporating the solubility and the dissolution of the drugs have been used to describe the PK data following pulmonary administration and to support the estimation of dissolved drug concentrations in lungs available for activity. To this end, we propose that biopharmaceutical pharmacometric models — i.e. semi-mechanistic pharmacometric models taking into account the biopharmaceutical properties of the formulation — can serve as a mechanistic framework to integrate all available knowledge, including the understanding of the formulation, the biology of the system and the pharmacology of the drug, with predictive capacity supported by the available data. These models can provide another means to evaluate drug delivery strategies for formulation development. With the ever-increasing computing power and availability of data, these modelling and simulation approaches have the potential to be further utilised and play a more important role in the development of inhaled pharmaceuticals.

**Concluding Remarks and Future Perspectives**

Inhaled drug delivery is intrinsically complex and variable. The efficacy of inhaled therapies is dependent on many factors including the properties of the compound, the performance of the formulation in a suitable device and the readiness of the consumer to use the product as instructed. While in vitro aerosolisation measurements of inhaled formulations are routinely performed, a clear relationship between APSD and clinical efficacy remains to be elucidated. Studies specifically designed to understand APSD and clinical efficacy would therefore be beneficial. In lieu of an empirical approach, an advanced understanding of particle deposition in relation to inhaled drug delivery is much advantageous. In that regard, various in vitro testings, ex vivo models, in vivo studies and imaging techniques have been developed for the study of particle deposition and, subsequently, absorption and disposition of drugs from the respiratory tract.

In spite of the recent advancements in the field, compared to drug delivery systems for more conventional routes of administration, the understanding of inhalable formulations remains relatively limited. In particular, the establishment of IVIVC for inhalable formulations remains to be a challenge. With the increasing interest in inhaled drug delivery, the development of more realistic and biorelevant in vitro models for formulation assessments would be beneficial. For instance, assays with improved designs in mimicking the airway epithelium and the lining fluids in different regions of the respiratory tract could be helpful for the study of drug absorption and disposition after regional delivery of the formulation. The results of these assays could, when combined with APSDs, better characterise and compare the performance of formulations.

Recent reports on the applications of various computational techniques in the development of inhaled pharmaceuticals.
mechanics of the consumer on inhaled therapies. In addition, the combination of MPPD modelling and APSD studies could greatly enhance the interpretability of APSD results by translating them into regional deposition patterns. Such application could then provide a mechanism to bridge the gap between APSD, deposition pattern and clinical efficacy of inhaled therapeutics. More recently, the application of machine learning to predict formulation performance using input variables from the API, the formulation and the device has also been shown to have potential and may be further explored.

Studies using other quantitative and modelling approaches such as PBPK and pharmacometric models for the development of inhaled therapeutics have also been reported. Depending on the specific purposes, building an applicable model could be challenging with the data and resources available. In particular, comparing to plasma concentrations, the sampling of lung concentrations is a challenging with the data and resources available. In particular, providing a mechanism to bridge the gap between APSD, deposition mechanics of the consumer on inhaled therapies. In addition, the application of machine learning to predict formulation performance using input variables from the API, the formulation and the device has also been shown to have potential and may be further explored.

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