Expert Opinion

Assessment methods of inhaled aerosols: technical aspects and applications

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The pulmonary route has been used with success for the treatment of both lung (asthma) and systemic diseases (diabetes). The fate of an inhaled drug (absorption and deposition) within human lungs has great importance, particularly in drug development and quality control. This article focuses on the various methods that are now applied for aerosol fate investigation. Several assessment methods, ranging from *in vitro* assays (impaction and optical systems) to *in vivo* experiments (imaging and pharmacological methods), are described. *In vitro* assays measure particle size distribution and emitted drug dose, which could be predictive of lung deposition pattern *in vivo*. However, *in vivo* methods provide direct information about the concentration and the location of inhaled drug within lung. Advantages and limitations of the different techniques are identified. In addition to these experimental techniques, mathematical deposition models, elaborated in more realistic conditions and designed to predict the fate of inhaled particles, are also illustrated.

Keywords: aerosol, assessment methods, drug delivery, gamma camera, impactor, lung, mass median aerodynamic diameter, mathematical simulation, pharmacological methods, pulmonary

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1. Introduction

During the past decade, the pulmonary route has been widely used for drug administration. It has been considered as an interesting way for the treatment of local pulmonary diseases (e.g., asthma, chronic obstructive pulmonary disease, cystic fibrosis [1], lung cancer [2]). Recently, inhalation has been recognised as a promising alternative for systemic disorders treatment (e.g., diabetes [3]). The lung has several advantages over other routes (oral, parenteral). It includes a rapid effect, specific targeting site, a reduced dose with equivalent therapeutic response, lower systemic side effects [4], the evasion of first pass hepatic metabolism [5] and an enhanced efficiency of the delivered drug.

The pulmonary route is constantly being investigated for the purpose of improving drug targeting and hence optimising aerosol efficacy. To be effective, pharmaceutical aerosols have to be deposited in the targeted site at a therapeutic dose within the lung. The aerodynamic diameter (mass median aerodynamic diameter [MMAD]) and physical characteristics of aerosol particles are considered as key factors affecting the regional drug deposition of inhalable particles in the lungs and thus conditioning the therapeutic efficacy. It is believed that in order to reach the lower respiratory tract and to optimise pulmonary drug deposition, aerodynamic diameters have to be between 1 and 5 µm [6]. Particles > 5 µm are usually deposited in the oropharynx, from which they are easily removed. However, particles sized < 0.5 µm are still moving by Brownian motion and settle very slowly [7].

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In reality, though, particle distribution pattern within the lung and therapeutic efficacy of aerosols is a very complex issue and depends strongly on the dynamic interactions of three classes of parameters: aerosol (aerosol formulation, the drug itself as an active pharmaceutical ingredient, the delivery device etc.); respiratory tract (anatomy, geometry, breathing pattern: flow rate, tidal volume etc.); and the patient (age, gender, disease status etc.) [8,9].

Several instruments and different approaches have been used for the assessment of aerosol particle generated by inhalation devices with a view to predicting their fate through the respiratory tract. *In vitro* characterisation methods provide information about the size distribution and the emitted dose of inhaled drug. It includes the optical methods, a rapid tool for particle sizing based on laser diffraction techniques, and the impactors, which determine the dynamic behaviour of particles by inertial impaction and allow a quantitative estimation of active pharmaceutical ingredient (API). *In vivo* experiments including imaging techniques and pharmacological analysis offer better prospects for quantifying lung deposition as they provide information about the amount and location of the drug delivered and absorbed after inhalation. Imaging techniques permit a visualisation of aerosol particle distribution within the respiratory tract and pharmacological assays quantify the drug concentration. Correlated with scintigraphic data, pharmacological measurements are useful for evaluating total and regional aerosol deposition. In addition to these experimental techniques, theoretical deposition approaches, designed to model and then predict the fate of inhaled particles in the lung, have been elaborated in more realistic conditions and widely applied.

The purpose of this review is to describe the principle of these theoretical and experimental assessment methods. Examples of application studies are given to emphasise the performance of such methods for aerosol fate investigation. Thus, key attributes and practical limitations are illustrated.

2. Anatomy and pulmonary deposition mechanisms

As the relative contributions from the large airways to the alveolar space are important factors concerning the local and systemic availability of target compounds, the sites and mechanism of deposition within the lungs need to be known.

2.1 Anatomy

The respiratory system is divided into the upper respiratory system (mouth or nose, pharynx, larynx) and the lower respiratory system starting from the trachea [10]. The airways in the human lungs can be broadly classified into two main groups: conducting airways (the trachea, bronchi and bronchioles), whose function is to conduct and transport air from the outside and deliver it to the respiratory airways – the alveoli; and the site of diffusion of gases for oxygen exchange with the blood [10,11].

The respiratory tract is lined entirely by a continuous layer of epithelial cells. The airway epithelium comprises at least six distinct cell types: ciliated cells, mucus goblet cells, Clara cells, serous cells, basal cells and dense core-granulated cells [12]. The relative distribution and abundance of the epithelial cell types vary significantly within different levels of the lung between the trachea and alveoli [13]. Ciliated cells (50% of the epithelial surface) are the most abundant cells at all levels of the airways; their main function is the propulsion of mucus in the proximal direction and out of the lung (mucociliary clearance) [14]. In the bronchial pseudostratified epithelium, the ciliated cells are interspersed by secretory cells, mainly mucus-secreting goblet cells. In the bronchiolar cuboidal epithelium, the ciliated cells are interspersed mainly by Clara cells [12]. The alveolar squamous epithelium is composed of two types of pneumocyte: type I and type II pneumocyte alveolar cells. Alveolar type I cells represent the principal type (95%) lining the luminal surface of the alveoli [15]. Alveolar type II pneumocytes, also present in the alveoli (5%), are cuboidal secretory cells and possess microvilli [15,16].

All the epithelial cell types are capable of contributing to the airway secretions, which form a bilayer of fluid protecting the epithelium surface [17]. Respiratory secretions consist of mucus, pulmonary surfactant (PS) and periciliary fluid (thin-layer fluid interposed between the mucous layer and the epithelium) [18].

The mucus is the secretory product of mucus gland, goblet and epithelial cell secretions [14]. It is a non-homogeneous, adhesive, viscoelastic fluid containing glycoproteins, proteins and lipids in a watery matrix [19]. The mucus is transported out of the lungs by the ciliary motion of the ciliated cells lining the airways and by airflow [20]. Thus, airway mucus is part of the mucociliary clearance (MCC), also known as the mucociliary escalator. MCC is defined as the primary non-specific defence mechanism for the removal of secretions and foreign inhaled particles that have been deposited in the airways. The principle of the system is simple: the ciliated cells in the epithelium transport the mucus with deposited particles in a proximal direction and eventually the mucus is expectorated or swallowed [21]. It also provides a vehicle for the removal of alveolar macrophages and inhaled particles [20]. With respect to drug inhalation, aerosolised drug deposited in the lung will either penetrate the mucus and become absorbed or follow the mucus and eventually become swallowed or cleared [21].

The PS is the secretory product of alveolar type II pneumocytes. It is a complex mixture of lipids and proteins that forms a film at the hydrated lung epithelium–air interface. The PS exists in two major compartments in the lung: the intracellular or storage compartment in the cytoplasm of alveolar type II cells and the extracellular compartment in the alveoli and distal airways [22]. Under physiologic circumstances, these two fractions are maintained relatively equilibrated [22]. The chemical composition of PS is ~ 90% of its mass

phosphatidylcholines (PC) and 10% of protein fraction with two hydrophilic (Surfactant Protein SP-A, SP-D) and two hydrophobic apoproteins (SP-B, SP-C) [23,24]. The surfactant carries out two distinct functions. Lining alveoli, it reduces surface tension at the air–hydrated lung epithelium interface (prevents collapse during expiration) and plays a role in host defence against infection and inflammation (immune functions) [25-27]. This role relies on the nature of SP-A and SP-D [28].

2.2 Aerosol deposition mechanisms

Understanding of the principal mechanisms leading to inhaled particle deposition within the lungs is a key step in designing and testing medical aerosols. Three main physical processes governing the deposition of therapeutic aerosols have been described in the literature [29]: diffusion by Brownian motion, sedimentation by gravitational force and inertial impaction [30-32].

For larger particles (size > 3 µm), inertial transport is an effective transport mechanism. Deposition resulting from impaction increases with particle size, particle density and velocity in the airways [33]. It is most likely to occur in bifurcations of extrathoracic and upper bronchial airways. Sedimentation by gravitational force (size ranging from 3 to 1 µm) governs the particle deposition in lower bronchial airways and alveoli. It increases with increasing particle size, increasing residence time in the lung and decreasing airspace size [33]. It becomes negligible for particles < 1 µm, which are deposited in small airways and alveoli owing to Brownian motion (diffusion). Total deposition by diffusion therefore decreases with increasing particle size and becomes negligible for larger particles [33]. As therapeutic aerosol particles usually have an average diameter ranging from 1 to 10 µm, the Brownian motion has no significant effect on drug deposition [33].

The fate of inhaled particles in the respiratory tract is very complex and depends on the dynamic interactions of three classes of parameters: aerosol characteristics (aerosol formulation, the drug itself as an active pharmaceutical ingredient, the delivery device etc.); respiratory tract, most importantly the breathing pattern (flow rate, tidal volume etc.); and the patient (age, gender, disease status etc.) (**Figure 1**). Extensive discussions of these parameters can be found in reviews concerning particle deposition [34-36].

Particle size is the most important factor that influences deposition. It will influence both the site of deposition and the mass of inhaled drug that deposits in the respiratory tract. It is well recognised that the fate of aerosol particles is primarily determined by the MMAD. The MMAD of the particles is defined as the diameter of a sphere of unit density (1 g/cm^3) that has the same aerodynamic behaviour as the particle under consideration [30,31]. The regional deposition of inhaled particles as a function of their diameter is shown in **Figure 2**. For therapeutic aerosols, particles > 10 µm cannot enter the lungs and are already deposited in mouth/nose, pharynx and larynx by impaction. The larger the particles and the higher the air flow, the more efficient is the deposition by impaction, therefore the number of particles reaching lung periphery decreases. Particles between 0.1 and 1 µm in size are not well deposited in the lungs and a high fraction is usually exhaled.

3. Assessment methods of lung drug deposition

3.1 *In vitro* methods

The pulmonary deposition of aerosol particle is a strong function of particle size (MMAD) and emitted dose of API. A variety of *in vitro* techniques including optical (laser diffraction), and impactions methods (impactors) have been described for pharmaceutical aerosol assessment. These techniques yield parameters such as particle size (geometric or aerodynamic diameters), fine particle fraction (FPF) and emitted drug dose (uniformity of delivered dose [UDD], fine particle dose [FPD]). Thus, the various methods involving phase Doppler and time of flight, although of great value in aerosol characterisation, are not included.

3.1.1 *Laser diffraction analysis*

Optical instruments based on laser-light diffraction, correctly named 'low angle laser light scattering', have become a common technique for aerosol particle sizing [37]. The use of the laser diffraction technique (LD) for particle size distribution (PSD) in nebulised aerosol clouds dates from the 1980s [38]. LD analysis is based on the simple principle that in a laser beam, particles scatter laser light at angles that are inversely proportional to their size (large particles scatter at small forward angles, whereas small particles scatter light at wider angles). By the use of a Fourier transform lens, the diffraction pattern is focused onto a photodetector array at the focal plane of the optics. Two approaches are commonly used as an optical model to convert the light energy diffraction pattern into a size distribution: Fraunhofer theory described light diffraction around the particle; and Lorenz–Mie theory, which adds the effects of light refraction (through the particle) and light absorption or reflection (by the particles) [39].

LD instruments, commonly used for medical aerosol sizing, include the Malvern 2000, 2600, Malvern MasterSizer E, S and X, Malvern Spraytec® (Malvern Instruments, Inc., USA), and also the Sympatec (Sympatec, Inc., USA). The Malvern instruments apply Mie scattering, whereas the Sympatec spray analyser allows for the option of Fraunhofer or Mie approximations to derive the PSD [40]. Several studies of medical aerosol are using LD for a direct and rapid particle sizing. It has been concluded that this technique offers the advantages of reliability, accuracy, high resolution and high reproducibility [41,42].

3.1.2 *Impactors*

Inertial impaction is the most widely used sizing technique carried out for the *in vitro* determination of the PSD of aerosols from medical inhalers, in product development, batch release and in devices evaluation [43]. There are two classes of

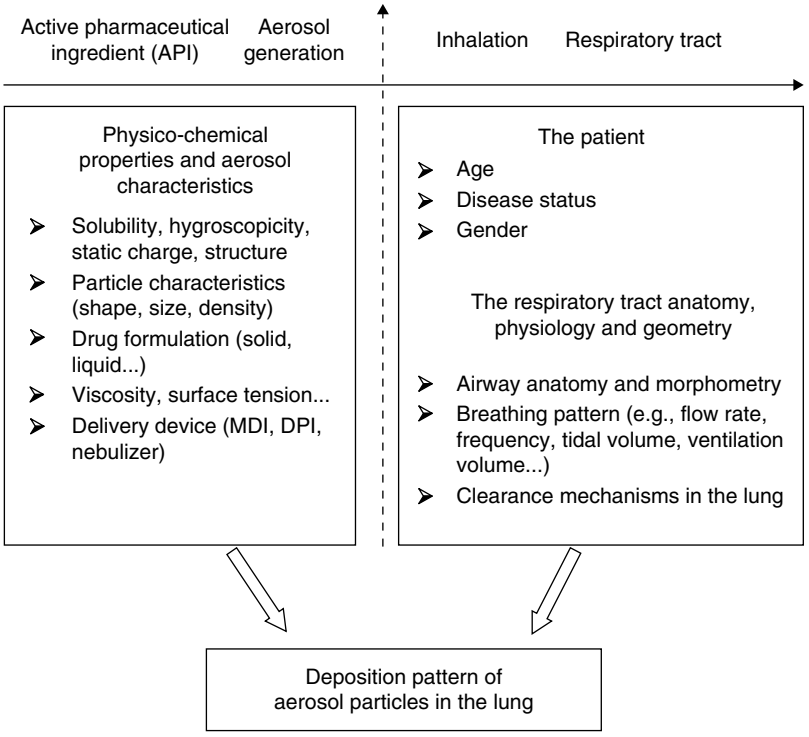

Figure 1. Parameters affecting aerosol particles deposition pattern within human lungs.

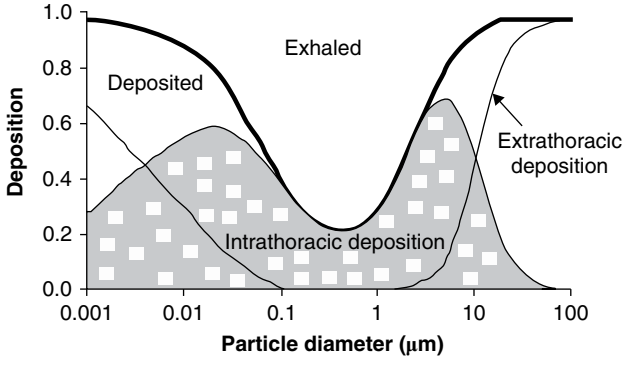

Figure 2. Deposition efficiency in the respiratory system as a function of the particle size.
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inertial impactors: single stage impactors and multiple stage impactors (also called cascade impactors). The cascade impactor (CI), initially described in 1945 was the oldest apparatus used to assess pharmaceutical aerosols based on inertial impaction. These devices present multiple stages with a set number of nozzles of decreasing diameter and a collection plate below each stage [44]. A vacuum pump, adjusted to generate a predesigned volumetric airflow rate, draws sample-laden air through the different stages. The principle is based on the aerodynamic behaviour of aerosol particles on the conveying air stream [45]. The airflow is deflected when its path is obstructed by the collection surface, then small particles with low inertia move around the surface and follow the air stream; however, large particles with high inertia will continue in the air stream and impact on the collection surface (**Figure 3A**). As the orifice size decreases, the linear velocity of air and particles' inertia increase at each stage, increasing the probability of particle deposition [44]. The reliability of impactors encouraged the regulatory authorities (the Food and Drug Administration [FDA] and Pharmacopoeias) to recommend their use for testing inhalation formulations and devices. Impactor specifications and standard test methods are included in European Pharmacopoeia EP [46] and United States Pharmacopoeia USP [47].

Impactors yield parameters such as MMAD and geometric standard deviation (GSD). GSD is a measure of the dispersion of particle diameters and can be defined as the ratio of the median diameter to the diameter at one standard deviation from the median diameter [48]. The experimental MMAD and GSD can be derived from the log-probability scale plot of the cumulative fraction of API versus cutoff diameter (**Figure 3B**). The MMAD is defined as the particle size at which the line crosses the 50% mark and the GSD can be estimated as [49]:

$$GSD = \sqrt{d_{84.1} / d_{15.9}}$$

where $d_{15.9}$ and $d_{84.1}$ are the sizes corresponding to the mass-percentile values of 15.9 and 84.1%. In addition, an

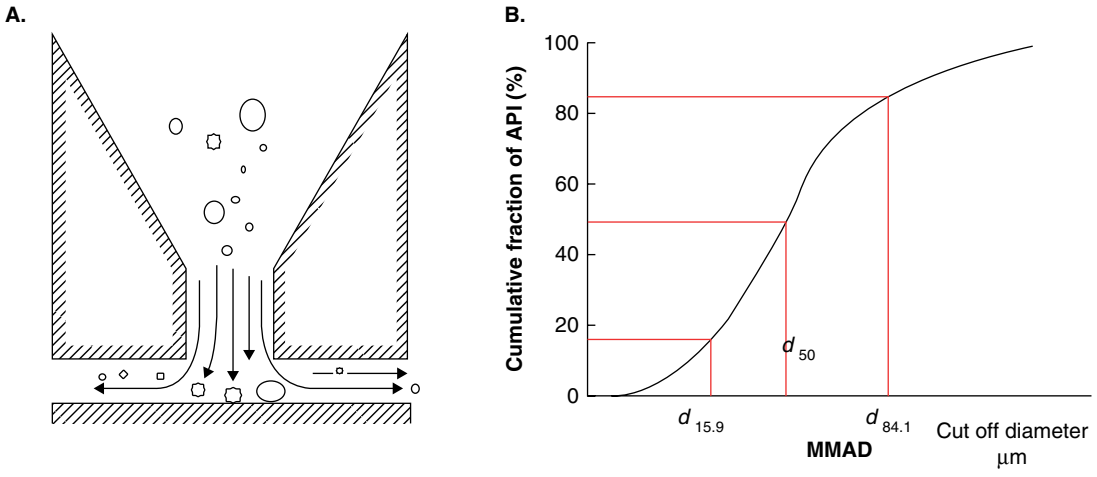

Figure 3. A. Schematic diagram of aerosol particles collected by inertial impaction. Large particles impact on the impaction plate whereas small particles are diverted and follow the airflow. **B.** Example of impactor collection efficiency curve.

impactor allows quantitative analysis of aerosols (mass distribution) estimated by the FPF and FPD [50]. The FPF and FPD have particular relevance, as they express, respectively, the percentage and the dose of drug that can be deeply inhaled and is theoretically available for pharmacological activity. The FPF may be defined as the percentage of the aerosol mass recovered from the lowest stages of a cascade impactor or as the percentage of the aerosol mass contained in particles with an aerodynamic diameter within a predetermined size range [51]. The EP specifies the upper size limit of 5 µm in aerodynamic diameter for FPF [46]. By contrast, the USP recognises that FPF range limits depends on formulations/devices being tested so there is no need to fix a limit [47]. In practice, the upper size limit for FPF is set at either 5.8 or 4.7 µm, corresponding to two cut-point sizes of the widely used Andersen cascade impactor (ACI) at 28.3 l/min [43]. Otherwise, the FPD is defined as the mass of API with an aerodynamic diameter < 5 µm. It may be calculated by interpolation from a log-probability plot of cumulative mass versus cutoff diameter of the respective stage of impactor or as the product of the total emitted dose from the inhaler and FPF [43].

Several types of impactor have been designed and operated; the most widely used instruments are the ACI, multi-stage liquid impinger (MSLI) and Marple Miller impactor (MMI) [51]. Recently introduced in pharmacopoeias, the next generation impactor (NGI) was developed to improve the size characterisation efficiency by inertial impaction [47]. The NGI was developed as a device that is simpler and less labour-intensive than the ACI. This planar seven-stage impactor, with an operational pre-separator at the inlet for oversized particles and a micro-orifice collector at the exit to collect the finest particles, has been designed specifically for studying both dry powder and metered dose inhalers (DPI and MDI, respectively) [52]. NGI provides physiologically appropriate stage cutoff diameters and possess tear-shaped cup impaction plates. Theoretically, this design should allow more accurate PSD determination of the delivered doses from medical nebulisers.

With recent interest in electrostatic characterisation of inhaled particles, the electrical low pressure impactor (ELPI), an electrical impactor that was originally designed for industrial and environmental aerosols, has been adopted for pharmaceutical application. ELPI is selected as the standard instrument for studying the relationship between net charge and particle deposition [53]. As there is no specific method for pharmaceutical aerosol, the design of the electrical next generation impactor (eNGI), which simultaneously measures particle size and charge distribution, is a first step for the development of a pharmacopoeia method for electrostatic characterisation of inhalational formulations [53].

Numerous studies compared data obtained using the NGI with those generated with other impactors and optical methods in order to confirm the particle sizing performance of this new pharmacopeia-approved CI (**Table 1**). The main characteristics of impactors, including the theoretical cutoff diameter, are summarised in **Table 2**.

In spite of the simplicity, reliability and good agreement between impactors in MMAD and mass distribution determination; PSD measurements by CI can be affected by several factors. Results variability depends on the operator/analyst and on the impactor (accessories, operating and environmental conditions) [54]. In practice, performing the CI measurement requires several manual operations, such as impactor assembly, connections to vacuum valves and pump, adjustment of air flow rate, inhaler manipulation and actuation, and quantitative assay of the deposited API. Human involvement in these steps, both because of imperfect technique and as a result of unintentional mistakes, increases the likelihood of bias and variability unless adequate measures are included in the method [54]. Otherwise, sources of impactor-related variability, including the associated equipment (the pump,

Table 1. Comparative studies on impactors.

Device/drug formulation	Results	Ref.
MDI Flixotide® (fluticasone propionate), Ventoline® (salbutamol sulphate) and QVAR® (beclomethasone dipropionate) CI: ELPI + NGI + eNGI	The difference in estimated MMAD and GSD for all MDIs using the NGI and eNGI were not found to be statistically significant The mean charge profiles from the ELPI and eNGI overlap well where most of the impacted doses were collected The eNGI is comparable to the NGI in measuring particle size distribution, while still being comparable to the ELPI in measuring charge distribution	[53]
Sodium fluoride (NaF; 2.5%) and generic albuterol (0.083%) the Omron MicroAir vibrating mesh CI: NGI + ACI TOF analysis = TSI 3321 APS	The results were more consistent and generally equivalent when determined by NGI and ACI analysis PSD determination by TOF in the APS was larger than results obtained by impactors	[41]
Vanceril® = MDIs containing beclomethasone dipropionate CI: NGI + ACI	Small differences in PSD profiles still existed to support NGI's design claims for reduced 'overlap' in the stage collection efficiency curves	[96]
Combivent® = MDI (anticholinergic bronchodilator, IB, and the b2-adrenergic bronchodilator, AS) CI: NGI + ACI + pre-separator + 1 l glass chamber or USP throat	Under their normal operating conditions, the ACI (28.3 l/min) and the NGI (30 l/min) yield similar PSDs and dose delivery profiles The particle bounce and re-entrainment effect is observed with both the ACI and the NGI and increases with increasing the flow rate The rough NGI collection cup bottoms are less sensitive to particle bounce and re-entrainment than the smooth surface of the ACI Collection performance degrades less for the NGI than for the ACI at higher flow rates 60 l/min	[97]
MDI Sizing instruments: ACI, NGI, and model 3321 APS	Fair agreement between the different methods for the aerodynamic assessment. Discrepancies between the techniques may be explained by particle bounce, incomplete evaporation of volatile constituents or the presence of surfactant particles	[43]

ACI: Andersen cascade impactor; APS: Aerodynamic particle sizer; CI: Cascade impaction; ELPI: Electrical low pressure impactor; eNGI: Electrical next generation impactor; GSD: Geometric standard deviation; MDI: Metered dose inhaler; MMAD: Mass median aerodynamic diameter; NGI: Next generation impactor; PSD: Particle size distribution; TOF: Time-of-flight.

induction port, which affects strongly the proportion of the emitted mass entering the impactor, air leakage, flow rate control etc. [54]) and environmental factors (temperature [55,56], relative humidity [57,58], particle evaporation [59] and coating of cups impaction plates [60]), present a significant influence on impactor performance and thereby particle size estimation. Then special attention to details that affect CI performance should always be *a priori*ty to optimise the measurement obtained.

Moreover, relevant practical drawbacks and limitations of CI analysis have been reported in the literature (**Figure 4**) [42,43]. It is an invasive, labour-intensive and time-consuming technique. Thus, impactors cannot be considered as a lung simulator; the geometry at the point of impact, collection surface hardness and coating and operation at constant flow rate do not correspond to physiologic conditions.

3.1.3 *Comparison of LD and CI*

Several comparative studies (**Table 3**) of LD and CI techniques have been published. Data show good correlation between these two techniques and prove that LD can be useful as an alternative or complimentary to inertial impaction techniques, but never as a substitute.

Impactor measures the MMAD (e.g., aerodynamic diameter), whereas LD measures geometric diameter. Aerodynamic diameter is related to the geometric diameter through the equation [61]:

$$\sqrt{C_{ae}} \times D_{ae} = D_v \times \left[\frac{\rho \times C_p}{\chi \times \rho_0}\right]$$

For spherical particles, Cunningham slip correction factors (C_{ae} and C_p) are close to unity and the dynamic shape factor χ is unity [61], then D_{ae} can be directly correlated to the D_v using the equation [62]:

$$D_{ae} = D_v \times \sqrt{\rho}$$

LD will therefore agree with aerodynamic techniques when measured particles are spherical (density = 1). For non-spherical particle or when density is lower than unity (e.g., porous particles), the D_{ae} will be smaller than D_v. As it assumes that all the particles are spherical, LD analysis, very

Table 2. Characteristics of impactors commonly used to size analysis medical aerosols.

Impactor type	NGI	ACI Model MARK II	MMI Model 150	MSLI
	Seven-stage cascade impactor	Eight-stage cascade impactor	Five-stage cascade impactor	Five-stage cascade impactor
Aerodynamic cutoff diameter (µm)				
$d50\sqrt{C_c} = \sqrt{\left[9\eta\pi D_j^3 (\mathrm{Stk}\,50)/_{4\rho p \times Q}\right]}$				
where d_{50} is the cutoff diameter, C_c is the Cunningham correction factor, η is the viscosity, D_j is the diameter of the jet, Stk_{50} is the Stokes number at 50% collection efficiency, ρ_p is the density of the particle and Q is the flow rate of air [98]				
Flow rate	***30 l/min***	***28.3 l/min***	***30 l/min***	***60 l/min***
Stage 0	–	9	–	–
Stage 1	11.72	5.8	10	13
Stage 2	6.4	4.7	5	6.8
Stage 3	3.99	3.3	2.5	3.1
Stage 4	2.3	2.1	1.25	1.7
Stage 5	1.35	1.1	0.63	< 1.7
Stage 6	0.83	0.7	–	–
Stage 7	0.54	0.4	–	–
Particle size range:	0.5 – 15 µm	0.1 – 10 µm	0.5 – 10 µm	0.8 – 13 µm 0.8 – 25 µm (with throat)
Flow rate range:	30 – 100 l/min	28.3 or 60 or 90 l/min	30 l/min Other models (MMI *150P* 4.9 – 12 l/min; MMI *160*: 60 – 90 l/min)	30 – 100 l/min
GSD_{stage}	1.2	1.25 – 1.4	1.2	1.4 – 1.5
Pharmacophore USP	Apparatus 6 for MDIs	(No pre-separator) Apparatus 1 for pMDIs (pre-separator) Apparatus 3 for DPIs	Apparatus 2 for DPIs	Apparatus 4 for DPIs
EP	Apparatus E	Apparatus D	–	Apparatus C

ACI: Andersen cascade impactor; MMI: Marple Miller impactor; MDI: Metered dose inhaler; NGI: Next generation impactor; MSLI: Multi-stage liquid impinger.
Adapted from [43,46].

suited for nebulisers, will present some limitations for DPI characterisation [39,62]. Furthermore, this technique does not involve a mass quantification of API separately from excipients [39,42]. For more details, some specific advantages and limitations of these techniques are summarised in **Figure 4**.

3.1.4 *Dose collection apparatus*

The dose collection apparatus has been developed for the 'uniformity of delivered dose' pharmacopeia recommended test. This apparatus must be capable of quantitatively capturing the delivered dose, defined as the drug dose delivered from the inhaler to the patient. It consists of a filter support base with an open-mesh filter support, a collection tube that is screwed to the filter support base and a mouthpiece adapter to ensure an airtight seal between the collection tube and the mouthpiece. The inhaler has to be connected and used as directed in the instructions for patients, then the content of the apparatus is quantitatively collected and the amount of API determined with a suitable and validated analytical method. The test for UDD is carried out 10 times for each preparation and for each API. The apparatus specifications, procedure details and compliance conditions are described well in EP pharmacopoeia [46].

3.2 *In vivo* methods

Direct measurement of inhalable drug distribution and deposition within the lungs has been realised using several methods: imaging techniques (gamma scintigraphy) and pharmacological analysis. The therapeutic efficiency of aerosol is considered as a function of the dose deposited at the targeted site within the lung. This local bioavailability, rather than the systemic bioavailability after absorption, is

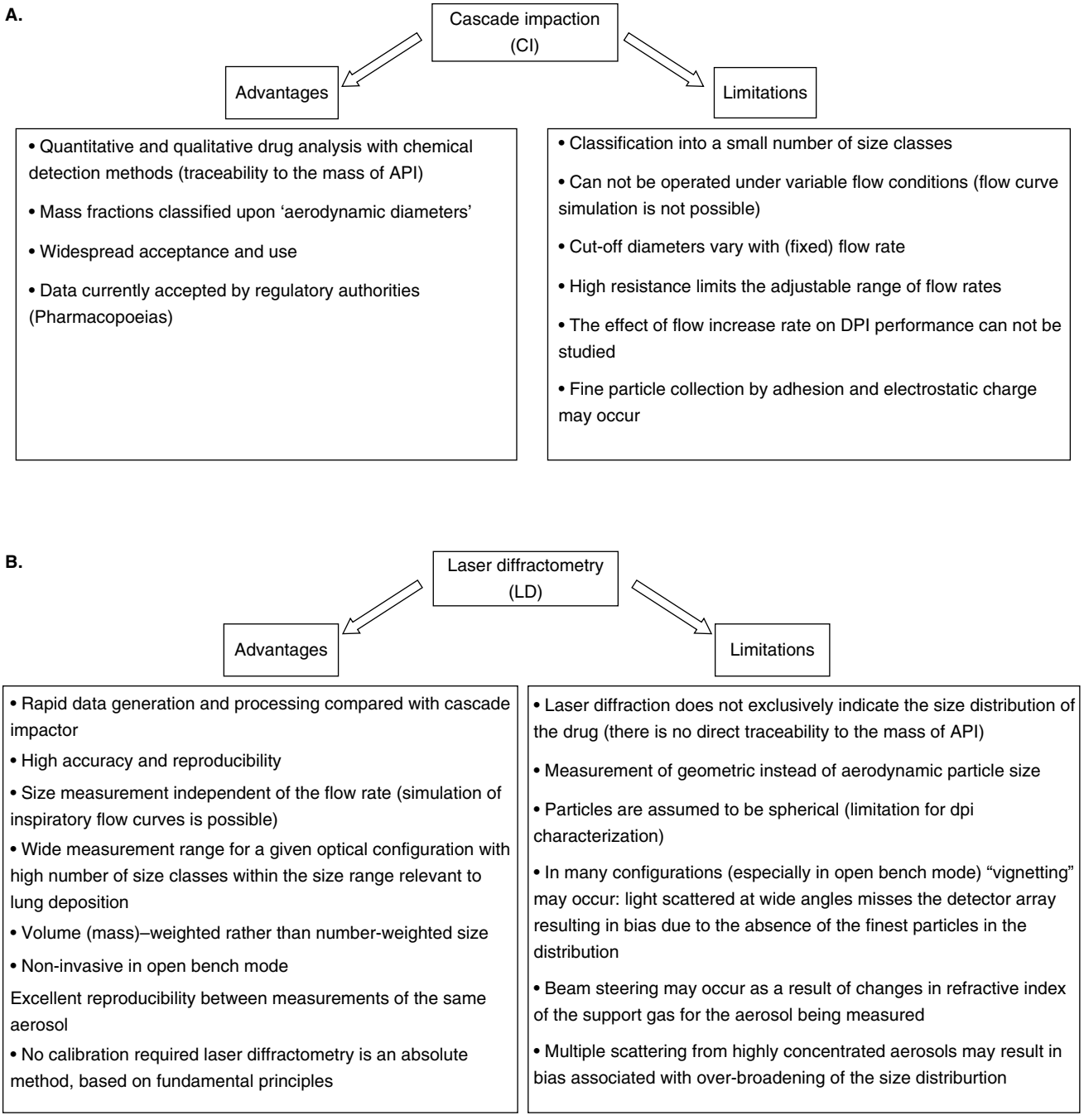

Figure 4. A review of some specific advantages and limitations of A cascade impaction and B laser diffraction.
Adapted from [39,43].

pertinent as it reflects the efficacy of drugs that act directly in the lung [63].

3.2.1 *Imaging methods*

In vivo imaging methods have been used extensively for assessing inhaled drug delivery since its first application. Two-dimensional gamma camera (planar gamma camera) is the most widely used imaging technique in pharmaceutical research [64,65]. It provides direct information about the quantity (percentage of the dose or mass of drug) and site of deposition of the drug in the lung after inhalation. However, the planar gamma camera provides only two-dimensional projection of the distribution and is thus very limited in distinguishing the distribution among airway regions and relating deposition to three-dimensional lung structure. More recently, pulmonary drug delivery has also been assessed by

Table 3. Comparative studies of LD and inertial impaction.

Methods	Conclusion	Ref.
Dry powder 'carrier-free' of tobramycin Inhalation devices (Aerolizer®, Spinhaler® and Handihaler®) LD: Malvern Mastersizer 2000® and Malvern Spraytec® CI: MSLI + NGI operating under Pharmacopoeia conditions	The Mastersizer 2000 permitted the determination of the size characteristics of individualised particles, whereas size results obtained from the Spraytec included the presence of some aggregates as it appears under the normal conditions of use of the inhaler by a patient The difference between these methods is the particle dispersion capacity Linear relationships and correlations ($R^2 > 0.9$) existed between the results obtained from, on one hand, the Mastersizer 2000 and the Spraytec and, on the other hand, the MSLI and the Spraytec regardless of flow rates and inhaler devices. The Spraytec could be a reliable technique for the development, evaluation and quality control of DPI	[99]
Five formulations: salbutamol base + 5 different sugar carriers Aerodynamic PSD was measured after aerosolisation at 28.3 l/min from the glass inhaler into an ACI and by LD at different flow rates	At 28.3 l/min a significant linear correlation was found between the fine fractions measured by LD and inertial impaction. The LD technique could be an important tool for particle size characterisation of DPI	[100]
Three different drug formulations Aerosol generator: Prototype Respimat® Soft Mist inhaler A comparison of 2 aerosol sizing methods: LD (Sympatec HELOS LD analyser) and ACI (Andersen Mark II 8-stage) at different experimental conditions (flow rate, relative humidity RH)	Good correlation between the ACI and LD The linear correlation of the cumulated fractions yields for all formulations: $CF(ACI) = 1.61 + 0.99 \times CF(LD)$ with ($R = 0.996$) The humidity of the ambient air had the strongest influence on PSD for both ACI and LD The simultaneous determination of the droplet size distribution leads to a good correlation between the ACI and LD method only if the measurements were performed at RH of 90% The influence of the flow rate on LD was negligible, whereas for ACI, flow rate dependence is expected	[101]
Nebulisation of a sodium fluoride solution with conditions reproducing the European Committee for Normalization protocol Nebulisers: Pari LC1® and Microneb® third type of nebuliser NL9® was tested with the Marple personal Cascade Impactor model 298(A-MPCI) and Mastersizer-X LD (Mastersizer-X) TOF: APS CI: Andersen + A-MPCI + GS1-CI	There was no difference between the Mastersizer-X and the A-MPCI or between the GS1-CI® and the A-MPCI in terms of MMAD Comparison between the APS® and the A-MPCI showed a significant difference with the Microneb. GSD obtained with the A-MPCI were on average 10% greater than GSD obtained with the other apparatuses but the differences were not statistically significant. The LD is proposed for PSD in the context of the European standard, and the Mastersizer-X is particularly interesting for industrial practice in view of its simplicity and robustness	[102]
Nebulisation of salbutamol + sodium cromoglycate Nebuliser Pari LC Star LD: Malvern Mastersizer X (MMX) CI: ACI and the commercially available MPCI	MMAD obtained with the MMX were virtually identical to the MMADs measured with both impactors when cooled at 10°C with no significant differences in GSD. At ambient temperature, MMADs were smaller (18 – 30%) with impactor with a significantly larger sigma (g) ($p < 0.05$) compared with the MMX	[103]

ACI: Andersen cascade impactor; CI: Cascade impaction; DPI: Dry powder inhaler; LD: Laser diffraction; MMAD: Mass median aerodynamic diameter; MSLI: Multi-stage liquid impinger; NGI: Next generation impactor; PSD: Particle size distribution; TOF: Time-of-flight.

three-dimensional imaging methods, including single photon emission computed tomography (SPECT) and positron emission tomography (PET), which provide accurate and highly specific data about the three-dimensional intrapulmonary deposition pattern [65]. Guidelines relating to experimental aspects of scintigraphic studies have been published as part of a British Association for Lung Research (BALR) consensus statement [66].

Scintigraphic studies require the production of radiolabelled aerosol. Thus far, the ^{99m}Tc has been used exclusively to radiolabel drug formulations for SPECT and planar imaging [67]. This radionuclide has ideal radiation energy (140 keV) to use with a gamma camera. Its short half-life of 6 h coupled with a very 'clean' radiation emission profile (it contains few beta-particles) result in very low radiation doses [68]. Typical radiolabelling methods involving ^{99m}Tc adsorption as sodium pertechnetate onto the surface of drug particles in a DPI, or Tc-diethylene triamine penta acetic acid (DTPA) addition to nebuliser solutions, offer the advantage of keeping the drug molecule unchanged during the labelling

Table 4. SPECT and PET properties.

	SPECT	PET
Advantages	Higher resolution/planar gamma scintigraphy It allows intrapulmonary deposition patterns to be quantified that may relate more precisely to deposition in different anatomical regions of the lungs It may detect differences in regional lung deposition patterns that are missed by planar gamma scintigraphy High availability	Using physiological compounds The drug is isotopically labelled, no modification of its chemical structure Some organic isotopes (^{11}C, ^{18}F) are positron emitters, and they can be incorporated directly into the drug molecule by isotopic substitution PET scans allow regional kinetics of the drug to be observed The spatial and temporal resolutions of PET are higher than gamma scintigraphy Corrections for tissue attenuation and gamma photon scattering can be implemented reliably in PET Improved attenuation factor
Disadvantages	The cost of the SPECT ++ Needs greater technical facility than planar imaging. It takes longer (up to 30 min), during which time redistribution of the radioaerosol by mucociliary clearance, coughing, or absorption into the bloodstream may occur To avoid extra tissue attenuation by the arms it is necessary to keep them raised during the procedure, which may cause discomfort The amount of radioactive material and hence the radiation dose involved is some 20-fold higher than for planar imaging	The cost of the PET imaging system The short half-lifes of the most commonly used radionuclides (^{11}C, ^{19}F…) The difficulties of synthesising radiolabelled analogues of drug molecules (considerable development work and validation)

PET: Positron emission tomography; SPECT: Single photon emission computed tomography.

process [66]. For PET, drug has been firmly labelled with Fluorine-18 (^{18}F) or Carbon-11 (^{11}C) as positron emitter isotope [64]. Before performing imaging studies, *in vitro* validation experiments are essential if deposition has to be quantified accurately. *In vitro* validation must be undertaken to analyse the drug to radiolabel association, to check that the drug and radiolabel distributions match one another across the full range of particle size bands, and to demonstrate that the radiolabelling process does not alter the delivery characteristics of the formulation [69].

Imaging techniques are specially applied to evaluate the efficiency of central versus peripheral lung drug delivery. The ratio of peripheral to central lung deposition (P/C ratio) was calculated as an index of regional lung deposition [70]. To analyse this P/C ratio, defining the regions of interest of the lung is very important. The boundaries of the lungs can be delineated using a ^{99m}Tc transmission scan or ventilation scan using radioactive inert gas (^{133}Xe or ^{81m}Kr). Otherwise, the imaging process is significantly affected by a local attenuation of the gamma rays in their passage out of the body and correction has to be applied for quantitative imaging [67,71]. Several methods have been developed to determine the attenuation correction factors (ACFs): density mapping of computer tomography (CT) scanning, measurement of lung uptake of ^{99m}Tc macro-aggregated albumin or total chest gamma ray transmission analysis [71].

Imaging techniques have been described widely in the literature; the advantages and disadvantages of PET and SPECT have been particularly discussed (**Table 4**) in several papers [72-76]. In spite of potential advantages and enhanced performance with respect to planar gamma camera, SPECT and PET techniques had been used relatively little until the last few years. This limited application for the assessment of inhalable drug delivery may be caused by disadvantages and practical problems.

3.2.2 Pharmacological methods

3.2.2.1 Pharmacokinetic and pharmacodynamic methods

Pharmacokinetic-pharmacodynamic (PK-PD) methods have assumed an important role in improving the efficiency of pulmonary drug systems. Information gained using PK-PD methods from the *in vitro* assessment right up to the end of Phase II development can underpin proof-of-concept and ensure that drugs are pharmacologically optimised.

PK methods provide a valuable tool for investigating lung deposition and bioavailability, and hence the ways of optimising drug delivery [77]. PD data are considered to be the 'gold standard' for assessing the efficacy and safety of new inhaled drug products, and form the basis of the clinical trials package in regulatory submissions [50]. In a complementary manner to PK methods, PD studies evaluate therapeutic efficacy, measure adverse events and establish a dose–response relationship for side effects. Whatever the approach, the choice of the method depends on drug class and specific molecules [78].

PK methods based mainly on the quantification of drug concentration in blood or plasma may be a key predictor of

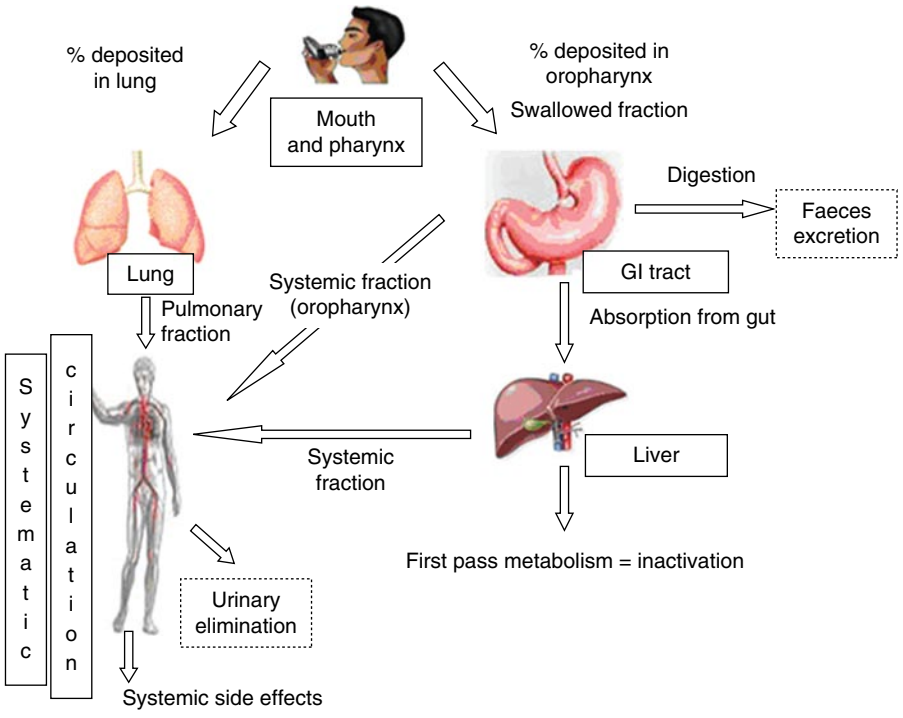

Figure 5. Schematic illustration of inhaled drug dose fate.

therapeutic response in the case of systemic drug effect, but they have limited relevancy to clinical efficacy of locally acting compounds [65]. Following inhalation (**Figure 5**), a fraction of the emitted dose is delivered to the bronchial tree (total lung dose) and will be either cleared by mucociliary clearance (and then swallowed and absorbed by means of the gastrointestinal [GI] tract) or absorbed through the airways epithelium. The other fraction is deposited in the oropharayngeal region and reaches the systemic circulation by means of local absorption in the oral cavity or after swelling in the GI tract followed by hepatic first pass metabolism [79]. Thus, drug enters the systemic circulation by the pulmonary and GI route but only the inhaled fraction of the dose provides the pulmonary therapeutic effect (effective lung dose) [80].

It is important to distinguish between the absorption from lungs and from the GI tract. If GI absorption of the drug is negligible and/or first pass metabolism is very high, it can be assumed that the plasma concentrations of these molecules after inhalation are entirely derived from pulmonary absorption, allowing precise characterisation of pulmonary bioavailability. Otherwise, according to drug molecules and metabolism, many PK techniques have been described to bypass this problem. It is achieved by the activated charcoal method (the oral administration of activated charcoal prevents the absorption of swallowed drug from the GI tract) or by exploiting the temporal shift between oral and pulmonary absorption (collecting plasma samples within a time window when only lung-derived drug is present) [81]. These techniques have been largely applied for assessing inhaled drug deposition (**Table 5**).

A PK and PD analysis of pulmonary effects after intravenous administration established the relationship between systemic and local action and enabled the prediction of pulmonary targeting [74]. The differences in the PK and PD characteristics of the inhaled drugs warrant careful consideration when used in clinical practice as they may result in differences in efficacy and safety profiles [82].

3.2.2.2 Pharmaco-scintigraphy

A combination of scintigraphy imaging and pharmacokinetic studies (called pharmaco-scintigraphy) can be very informative and valuable for systemically and locally acting products. Scintigraphy, especially SPECT and PET, provides direct information about regional aerosol distribution and pharmacology gives drug dose estimation, then combination offers a useful means of measurement of drug targeting by probing the relationship between targeted regional drug deposition profiles and systemic PK data [83].

Pharmaco-scintigraphy has been applied by Hirst *et al.* to assess the deposition of triamcinolone acetonide (TAA) and to determine the extent to which the Azmacort® spacer improves targeting of TAA to the lungs [83]. Ten patients with mild to moderate asthma received in a random way 3 delivered doses of 75 µg TAA by means of MDI coupled to the Azmacort spacer, and 3 delivered doses of 230 µg TAA via the same device, but without the spacer. The deposition of TAA, labelled with ^{99m}Tc, was assessed by gamma scintigraphy. Mean lung deposition expressed as mass of drug was similar for each, but when expressed as percentage delivered

Table 5. Examples of application of pharmacokinetics methods.

Aim of the study	Method	Results	Ref.
The pulmonary bioavailability of inhaled BDP aerosols of different particle sizes	10 mild asthmatic patients inhaled monodisperse BDP aerosols with MMAD of 1.5, 2.5 and 4.5 µm A randomised single-blind crossover trial *Gastrointestinal absorption was stopped by activated charcoal* Plasma concentrations of 17-BMP were measured	Mean maximum concentrations (C_{max}) of 17-BMP of aerosols with MMAD: 1.5 µm : 475 pg/ml 2.5 µm: 1300 pg/ml 4.5 µm: 1161 pg/ml The area under the curve (AUC) values of 17-BMP for MMADs 1.5 µm: 825 pg/(ml h) 2.5 µm: 2629 pg/(ml h) 4.5 µm: 2276 pg/(ml h) The mean terminal half-life of 17-BMP for all three aerosol sizes was around 1.5 h	[81]
Comparative study of the lung deposition of BUD inhaled from Turbuhaler and FP inhaled from Diskus	15 patients aged 8 – 14 years Intravenous infusion of 200 µg BUD Intravenous infusion of 200 µg fluticasone dipropionate Inhalation of 800 µg BUD via Turbuhaler, inhalation of 750 µg FP via Diskus Inhalation of BUD and FP on the same day *Charcoal was ingested to eliminate drug uptake from the gastrointestinal tract* Plasma levels of BUD and FP were measured for 21 h on 5 separate days.	The mean lung deposition of drug after Turbuhaler and Diskus inhalation was 30.8% (BUD) and 8.0% (FP) when administered on separate days, 29.5% (BUD) and 7.6% (FP) when inhaled on the same day. Lung deposition is four times higher after inhalation from Turbuhaler than after inhalation from Diskus	[104]
The determination of the reproducibility and dose–response relationship for urinary salbutamol excretion post-inhalation	15 volunteers inhaled either 1, 2, 3, 4 or 5 doses of 100 µg salbutamol on separate days and then 7 of these also repeated each of the 1, 3 and 5 doses on 5 occasions After each study dose urine was collected 30 min post-inhalation	The 30 min salbutamol urinary excretion post-inhalation is linear with inhaled dose and reproducible This positive link to a clinical bioassay together with the reproducibility and dose–response properties highlights the potential of this 30 min index of lung deposition in bioequivalence	[105]
The relative lung and systemic bioavailability of SC following inhalation by different methods	On 3 separate randomised study days, 7 days apart, subjects inhaled 4 × 5 mg from an Intal MDI, 4 × 5 mg from an MDI attached to a large volume spacer (MDI + SP) 20 mg from an Intal Spinhaler (DPI). Urine samples were provided at 0, 0.5, 1, 2, 5 and 24 h post dose	The mean ratio of these amounts excreted in the urine over the first 30 min and the 24 h cumulative amount excreted over the 24 h post-inhalation are also estimated The results highlight better lung deposition of sodium cromoglycate from a MDI attached to a large volume spacer	[106]
The pharmacokinetics of inhaled (*R*,*S*)-albuterol in healthy human subjects	10 subjects (5 females and 5 males) inhaled 2 puffs (180 µg) of Albuterol via a MDI and spacer device *Charcoal slurries were ingested to block gastrointestinal absorption of drug* Venous samples were obtained from each subject at 13 time points from 0 to 12 h post-inhalation	The PK profiles conformed to a two-compartment extravascular model with first-order absorption kinetics. The parameters T_{max}: 12.6 ± 2.2 (SD) min C_{max}: 1469 ± 410 pg/ml $t_{1/2}$ of distribution: 17.9 ± 8.2 min $t_{1/2}$ of elimination: 4.4 ± 1.5 h	[107]

17-BMP: 17-beclomethasone monopropionate; BDP: Beclomethasone dipropionate; BUD: Budesonide; FP: Fluticasone propionate; SC: Sodium cromoglycate.

dose, lung deposition was higher for TAA with spacer (53.8%) versus TAA without spacer (26.0%), indicating superior drug targeting for TAA with spacer. Pharmacokinetic data showed higher plasma levels of drug for TAA without spacer, resulting from higher oropharyngeal deposition shown by gamma camera. The spacer reduced oropharyngeal deposition. Pharmaco-scintigraphic data proved that the addition of a spacer device to the actuator used for TAA delivery has significant benefits in terms of drug targeting, as oropharyngeal deposition is reduced, lung deposition expressed as percentage of delivered dose is increased and systemic exposure is reduced.

Cass *et al*. studied zanamivir deposition in the respiratory tract by imaging and pharmacokinetics techniques, after oral inhalation from two different devices [84]. Participants were given zanamivir dry powder formulated with ^{99m}Tc from the Diskhaler® or the prototype device on separate days. Scintigraphic images of the chest and oropharynx were recorded and the serum and urine concentration of zanamivir were measured after drug administration. Similar deposition data were obtained with the Diskhaler and the prototype device and pharmaco-scintigraphy was confirmed as being a reliable technique for measuring zanamivir deposition in the respiratory tract.

Lung deposition of new formulations of budesonide, using solid lipid microparticles (SLmP) as a pharmaceutically acceptable filler and carrier for inhalation aerosols, and administered from a DPI (Cyclohaler), were compared with that from Pulmicort Turbuhaler in a recent study [85]. Lung deposition was determined by gamma scintigraphy and pharmacokinetic assays. Six healthy volunteers took part in a three-way randomised crossover study, and inhaled a nominal dose of 400 µg budesonide, labelled with ^{99m}Tc, on each study day. The percentage of dose (SD) in the whole lung was 49.9% (3.7) for the lipidic matrix form and 62.8% (4.9) for the lipidic physical blend formulation. Furthermore, the relative drug availability obtained from the pharmacokinetic evaluation, expressed as the percentage of pulmonary absorption of the comparator product, was 154 and 220% for lipidic matricial form and lipidic physical blend form, respectively. The results of the present study indicate that pulmonary administration using SLmP gives a prominent and significant increase in budesonide lung deposition.

3.3 Modelling

Deposition efficiency of particles in various locations of the human lung depends mainly on lung geometry, breathing parameters and aerosol properties.

Controlling aerosol characteristics and breath pattern is possible in aerosol formulation and inhalation device conception, but clinical protocols used to determine lung morphology are expensive and not always feasible. To overcome this limitation, methods allowing simulation of lung airways have been developed and many pulmonary mathematical models have been elaborated. These models can predict regional deposition fraction of inhaled particles during a breathing cycle, and linked to biological outcome, an exposure dose–response relationship can be established [86].

3.3.1 *Classification*

A mathematical model describing deposition of aerosol particle in the respiratory tract involves three components: the geometry model of the lung, airflow profile and lung ventilation. Two broad categories are distinguished: empirical and mechanistic models [87]. Empirical models (EM) consider the human respiratory tract as a series of anatomical compartments through which the aerosol passes during a breath [87]. It is based on experimental data and summarised by mathematical formulae where lung geometry and the true breathing parameters are not included [88]. Such basic models, in particular the model recommended by the International Commission on Radiological Protection (ICRP), are attractive because they are simple and useful for quick calculation of particle deposition [89]. However, the application range of such models is strictly limited to the specific morphology, physiology and lung conditions for which the model parameters were adjusted. Mechanistic models (MM) can be classified in several ways [86]. They calculate respiratory deposition on the basis of a more realistic description of lung structure and physiology than empirical models, and may include physical processes affecting particles' behaviour in lung airways [88]. However, the precise description of the realistic lung morphology is very difficult, which is why most theoretical studies use the simplest model in structure, Weibel's symmetric lung model [90]; but in reality, the airway branches are not truly symmetric, and airway dimensions vary widely among the branches within the same level of branching generation [90]. With advances in measuring technique, refined airway models assessing the effects of morphological and inter-subject variability have become available in recent years [91]. Mathematical approaches have passed through many stages of model development (**Table 6**), ranging from simple one-dimensional models of the lungs with deterministic sequences of airway bifurcations, including 'Lagrangian dynamical models' (LDMs), which model some of the dynamical behaviour of the aerosol in a frame of reference that moves with the aerosol, and Eulerian dynamical models (EDMs), where dynamical behaviour of the aerosol in the respiratory tract is viewed by a stationary observer, to more complex stochastic lung models and three-dimensional models simulating particle behaviour in single, double and triple airway bifurcations [92].

3.3.2 *Application of mathematical models*

In the literature, numerous respiratory simulation models have been described supporting the increasing interest of predictive mathematical models in aerosol assessment. Regional aerosol deposition efficiencies have been widely evaluated using the ICRP 66 model, which summarises experimental data on aerosol deposition and clearance in man for various particle sizes and breathing patterns.

Table 6. Mathematical model evolution.

Model	Description
Weibel's Symmetric lung model	Airways assumed to be dichotomous branching system with 23 generations
ICRP 66 respiratory tract model [89]	NCRP (1997) and ICRP (1966) particle deposition models are used in risk assessment analyses for deriving percentages of radioactive material deposited in different regions of the lungs based on physiological and environmental factors A task group of NCRP developed a new respiratory tract dosimetry model to update the ICRP 66 model
Semi-empirical model Empirical models	Based on experimental measurement. Lung geometry and the breathing patterns are not included [91]
Mechanistic models [87]	Asymmetric airways for the tracheobronchial tree
	Multiple-path model
	Multiple-path with realistic lung ventilation model
	Typical path with a symmetrical lung geometry
Lagrangian model [108,109]	The Lagrangian approach tracks individual particles within the flow field and can account for a variety of forces on the particle including inertia, diffusion, gravity effects and near-wall interactions
Eulerian model [86]	Considers inert particles and deals primarily with effects connected with physiological and morphological attributes, rather than aerosol effects
	Including aerosol dynamical processes (growth, coagulation and thermophoresis) but ignoring axial dispersion

ICRP: International committee on radiological protection; NCRP: National council on radiation protection and measurement.

Dunbar *et al.* characterised therapeutic aerosol (placebo powder) by impaction (*in vitro*), imaging (*in vivo*) and mathematical simulation [93]. PSD characteristics were measured *in vitro* using the MSLI. *In vivo* lung deposition of labelled particles (^{99m}Tc) was obtained by gamma scintigraphy and was characterised by high reproducible emitted doses. Then aerosol lung deposition was predicted by calculating the extrathoracic fraction using the ICRP model. It was concluded that the ICRP model using the aerodynamic PSD measured by the MSLI provided a good estimation of the mean *in vivo* lung deposition.

The particle deposition pattern from two different nebulisers has also been estimated using the ICRP empirical model and compared with values obtained by experiment. Radiolabelled aerosols of different particle size were delivered to 12 human subjects [94]. Medical imaging was used to assess the deposition in the body. The results showed generally good agreement between model and experiment deposition data in the extrathoracic and conducting airways for both aerosols. However, there were significant differences in the fate of the remainder of the aerosol between the amount deposited in the alveolar region and that exhaled. The inter-subject variability of deposition predicted by the model was significantly less than that measured, for all regions of the body. The model predicted quite well the differences in deposition distribution pattern between the two aerosols. In conclusion, this study has shown that the ICPR model of inhaled aerosol deposition shows areas of good agreement with results from experiment. However, there are also areas of disagreement, which may be explained by hygroscopic particle growth and inter-subject variability (differences in the morphology and physiology of the oropharyngeal region) not included in this models. A stochastic empirical version of the ICRP66 respiratory tract model has been developed by Bolch. 2001 (LUDUC: Lung Dose Uncertainty Code) and was applied for polydisperse aerosols.

Balashazy *et al.* have quantified the local deposition patterns of therapeutic aerosols in the oropharyngeal airways, healthy and diseased bronchi and alveoli using computational fluid and particle dynamics techniques [92]. A user-enhanced computational fluid dynamics commercial finite-volume software package was used to compute airflow fields, deposition efficiencies and deposition patterns of therapeutic aerosols along the airways. Adequate numerical meshes, generated in different airway sections, enabled trajectories and local deposition patterns of inhaled particles to be defined more precisely than before. Deposition patterns show a high degree of heterogeneity of deposition along the airways, being more uniform for nanoparticles compared with microparticles in the whole respiratory system at all inspiratory flow rates. Both airway constrictions and the presence of tumors significantly increased the deposition efficiencies compared with the deposition efficiencies in healthy airways. In alveoli, the deposition patterns are strongly influenced by particle size. This study has demonstrated that mathematical modelling can be a powerful tool in the aerosol drug delivery optimisation.

The particle deposition in three idealised proximal lung bifurcation models with an idealised mouth–throat was investigated experimentally by Zhang and Finlay [95]. These

bifurcation models included a small symmetric bifurcation model, an intermediate asymmetric bifurcation model, and a large symmetric bifurcation model. Idealised mouth–throat geometry (the 'Alberta geometry') was used as the inlet to these bifurcation models. Monodisperse aerosol particles of DEHS (di-2-ethylhexyl-sebecate) oil with mass median diameters in the range 2.5 – 7.5 μm were used at steady flow rates of 30 – 90 l/min. Particle deposition was measured by gravimetric techniques. The results show that particle deposition in the mouth–throat and trachea accounts for the major portion of total deposition in the entire models used and particle deposition fraction in the proximal lung bifurcations is lower compared with that deposited in the regions upstream (the mouth–throat and the trachea). Total particle deposition efficiency increases on increasing either inertial parameter or Stokes number. Total particle deposition varies appreciably from model to model. The laryngeal jet is the key factor dominating particle deposition within the trachea. An effect of Reynolds number on particle deposition efficiency in the trachea is observed. In addition, particle deposition in the bifurcation region is influenced little by the upstream flow condition, therefore the effect of the laryngeal jet on deposition seemingly does not propagate to the bifurcations downstream.

4. Conclusion

Assessment methods of therapeutic aerosols can provide helpful information about the fate of inhaled drug within lungs and therefore perform a valuable role in the development of new aerosol formulation and inhaler devices. LD and inertial impaction are usually considered as the method of choice for PSD characterisation. Impactors have been used extensively for the determination of aerosol mass size distribution. Whole lung deposition may be measured by gamma scintigraphy or pharmacokinetic method. Then, for regional lung deposition pattern, a three-dimensional imaging technique (SPECT and PET) may be preferred. Otherwise, a combination of scintigraphy and pharmacology, in 'pharmaco-scintigraphic' studies, generated more specific information about aerosol targeting. Mathematical modelling of the respiratory tract has been elaborated to overcome some of the limitations associated with experimental methods and make possible an approximation of lung deposition using more realistic conditions. Each method presents many advantages but also some limitations and, in practice, the combination of characterisation techniques or correlation between data seems to be the key part of aerosol investigation.

5. Expert opinion

The fate of inhaled particles (deposition and absorption) within human lung has long been of great interest in the pharmaceutical industry as the respiratory tract offers a promising non-invasive route for both systemic and locally acting drug. In the literature, several assessment methods have been described and widely applied for aerosol formulation. Owing to a plausible link between aerodynamic behaviour and the inhaled particle's fate within the respiratory tract, the particle size (MMAD) was recognised as an important factor affecting therapeutic efficiency and safety of aerosol.

In vitro assessment methods include optical and inertial impaction analyses. Pharmacopoeias selected inertial impaction as the standard method for aerosol assessment. CI provides a direct and reproducible measurement of both MMAD and drug mass distribution, which are relevant to the deposition within lungs; but this technique is a complex method, time-consuming, and may be a source of error due to experimental conditions (flow rate effect and aerosol evaporation during the analysis), which affects the measurement quality. Furthermore, impactors cannot be considered either as breathing simulators or as lung models. LD is a rapid particle sizing method and comparative studies versus impactors showed good correlation between data generated with both techniques. It has also been established that in spite of its lack of specificity, LD offers several advantages over inertial impaction (simplicity, no flow rate effect and no evaporation affecting result quality). As *in vitro* data are seldom obtained in similar conditions to patient use, *in vivo* methods, which are carried out in physiologic and clinical context, offer more suitable results. Information about whole and regional lung deposition of inhaled drug can be measured by gamma and three-dimensional imaging techniques. Pharmacokinetic methods can be used to quantify drug delivery to the lungs and plasma levels, whereas any information about the regional pattern of deposition can be provided. Pharmaco-scintigraphy, correlation between PK (quantification of drug) and scintigraphy (regional pattern), improves the specificity and the precision of measurement. In other aspects, mathematical models of respiratory tract have been developed to have access to more realistic indicative information and improve data quality for aerosol investigation. These theoretical models can predict regional particle deposition during a breathing cycle, and linked to biological data, an exposure dose–response relationship can be established. Numerical simulations have more advantages over experimental method in providing detailed information of particle motion, particle deposition and gas flow field in the respiratory tract without resorting to costly experimental *in vivo* studies. Arguments for using mathematical models as a means of predicting aerosol fate have been raised; however, improved accuracy of data must be demonstrated before this approach is accepted by the medical community as a suitable and reliable method for aerosol assessment.

By giving complementary information about aerosol fate, no method can be selected without reserve for aerosol investigation, however, combining *in vitro* and *in vivo* techniques and correlating data is an important goal and may be a useful remedy to the complexity of aerosol assessment. At present there are few published correlation studies; the challenge in the future will be to establish a clear relationship

between *in vitro* and *in vivo* methods, especially mathematical simulation and pharmacological or imaging methods, and to develop new approaches so that optimum delivery patterns can be defined.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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