EXPERT OPINION: REVIEWING CURRENT THINKING ON THE IN VIVO BEHAVIOUR OF PARTICLES IN THE EXTRA-FINE REGION

The inhalation route is a fast and effective way of delivering medication, both locally to the lungs and systemically to the body. Conventional wisdom for the development of inhaled products is that the preferred particle size for drug delivery is one to five microns and it is typically suggested that particles less than one micron are exhaled, but is this true? In this review, David Lewis, PhD, Head of Laboratory, Chippenham Research Centre, Chiesi UK Ltd, reports the latest research on the changing view of the behaviour of particles in the extra-fine region and their potential for enhancing inhaled drug therapies.

Pulmonary drug delivery has generated a significant amount of interest in pharmaceutical research because of the lung's capacity to absorb pharmaceuticals for both local treatment and systemic delivery.[1] The large surface area and highly permeable air-to-blood barrier provided by the respiratory system make it a highly receptive site for drug delivery, most especially for the local, rapid and effective treatment of, for example, asthma and chronic obstructive pulmonary disease.

However, the development of inhaled drugs is complex, as to achieve targeted deposition three conditions must be met:

1) The inhaled aerosol formulation must be sized for drug deposition along the respiratory tract including in the deep lung
2) The drug delivery device and formulation must generate an aerosol cloud containing a high proportion of suitably sized particles
3) The deposition of the drug should translate into functional and clinical benefits.

Better understanding of these parameters and their interrelationship helps to maximise the benefits and efficiency of pulmonary drug delivery systems but the majority of such research has focused on the behaviour of particles within the 1-5 µm range, which are known to deposit successfully in the pulmonary region. Particles larger than 5 µm will typically impact on the oropharynx and be swallowed. Assuming a continuum of behaviour, with finer and finer particles taking longer to deposit, the majority of particles less than 1 µm in size are expected to flow back out from the body on the exiting breath. Currently particles less than 1 µm in diameter are therefore not considered to be of consequence for drug delivery.

TIME TO RE-EVALUATE?

Recent technological developments have seen a wide range of industries look in more detail at particles in the 1-2 µm and nano range, and new evidence is emerging to suggest that assumptions about inhaled behaviour may not be correct. Indeed, studies investigating the deposition sites of extra-fine and sub-micron particles have found that it is possible that these particles could in fact be effectively delivered to the small airways in the deep lung.[2]

Clinically this might not only aid the efficiency of drug delivery, but could also result in a more uniform localised therapeutic response, if the drug was deposited in both the central and peripheral airways.[3,4] A recognised trait in certain asthma sufferers is persistent small airway dysfunction and

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this seems to be associated with poor disease control. By targeting deep lung deposition extra-fine formulations of inhaled asthma therapies may therefore also have the potential to 'unlock the small airways compartment' and improve treatment efficacy.[4,5]

Furthermore there is evidence that when an aerosol is mainly composed of very fine particles the difference between *in vitro* aerosolisation and *in vivo* behaviour is less pronounced.[6] This suggests that aerosols with a finer particle-size distribution could exhibit behaviour that is relatively independent of flow rate, a feature that would enable the delivery of more controlled deposition across patient groups with different inspiratory profiles.

Closer scrutiny of the behaviour of extra-fine aerosols in terms of pulmonary delivery calls for data generated both *in vitro* and *in vivo*. Interpretation of such data can be used to answer the following questions and to provide greater insight:

- How do aerosols of extra-fine particles behave?
- How do extra-fine particles behave in the respiratory system?
- Are current *in vitro* and computer based particle behavioural models sufficient for describing this behaviour?

Answering such questions will help resolve the potential pharmacological impacts and consequences of delivery of extra-fine aerosols for treatments for a wide range of applications. If particles around one micron and smaller are not simply exhaled then there are possible implications in terms of the sites of deposition with the lung, the dose received by the patient and the therapeutic activity of the drug. Further, improved insights may also provide a firm basis for future advancement in inhaled nano-medicine strategies.

DEFINING EXTRA-FINE & SUB-MICRON AEROSOLS

The development and quality control of orally inhaled and nasal drug products (OINDPs) relies on making *in vitro* measurements that reflect the likely success of drug delivery and targeted deposition. These include assessing the total quantity of drug emitted from the device and therefore available to the patient, and the aerodynamic size of the particles that make up the emitted aerosol. This impacts the percentage of the total dose that reaches the lungs during inhalation, as well as its regional intrapulmonary deposition, and is therefore therapeutically active.

Inertial impaction, sedimentation and Brownian diffusion are all factors that are affected by particle characteristics and that influence the site of deposition in the airways. Influential particle characteristics include shape, density and, most especially, size. For inhaled product delivery, aerodynamic particle size measured by the technique of inertial cascade impaction, is the metric used as a primary indicator of deposition behaviour because of its relevance, which stems from a shared dependence on shape and density. The mass median aerodynamic diameter (MMAD), one of the most commonly used inhaled product metrics, defines the size of aerosol particles, taking into account their geometric diameter, shape and density.

In terms of aerodynamic particle size, which is equal to the geometric particle diameter for spherical particles or unit density, a sub-micron particle can be identified simply as a particle with a diameter of less than one micron.[1]

There is an increasing amount of research into extra-fine and sub-micron aerosols. However, as yet a definition has not been agreed. Aerosols can be either polydisperse, consisting of multiple particle sizes, or monodisperse, containing particles of uniform size. For a monodisperse aerosol to be sub-micron, the particles need to have an aerodynamic particle size of less than one micron. However, there are, in reality, no truly monodisperse aerosols – most contain a distribution of particle sizes. For polydisperse aerosols, it has not been formalised as to what percentage of the particles need to be extra-fine or sub-micron to merit the respective classifications.

Typically, when designing an inhaled delivery device, limits are set that define an optimal range for the MMAD of the particles and the fraction (based on mass or volume) of particles that can acceptably fall outside of this range. An evaluation of dry-powder inhalers (DPIs), conducted by Krishnaprasad, found that the majority of devices investigated, contained approximately 50% of particles within the fine particle fraction.[7] This suggests that many DPIs could already be delivering a proportion of the dose in the extra-fine range, even if the aerosol as a whole cannot be classified as extra-fine. This proportion can be quantified through analysis of the residual dose captured in the final filter of a cascade impactor.

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The term "extra-fine" is now used routinely to describe inhaled particles and has been featured in several studies, [8] but there remain discrepancies in the term's definition that cause some confusion and make studies harder to compare. General consensus sees particles less than 1-2 µm referred to as extra-fine but some papers use this same term to refer to particles less than 0.1 µm in diameter.[2]

For the purposes of this article, extra-fine particles are defined as those that have an aerodynamic particle size of less than 2 µm, to differentiate them clearly from particles that are traditionally defined as lying in the fine particle fraction. This figure has been selected on the basis of clinical relevance, it being the upper size limit of particles that are able to penetrate to the small airways of the lung.[9]

PARTICLE BEHAVIOUR IN THE LUNG

In pulmonary delivery, there are three factors that influence particle deposition behaviour:

1) The characteristics of the aerosol
2) The anatomy of the respiratory tract
3) The airflow patterns in the lung airways.[10]

Deposition is quantified in terms of the ratio of the mass of particles deposited in the respiratory tract to the mass of particles inhaled [11] and governed, as mentioned earlier, by the mechanisms of impaction, sedimentation and diffusion.

The mass of a particle affects its travelling velocity, which is also determined by the velocity of the respiratory airflow. Larger particles will tend to travel more slowly but also have greater inertia making them more prone to impaction. Gravitational deposition is dependent on residence time and particle settling velocity and is therefore promoted by larger particle size and the longer residence times that more easily occur in the small conducting airways and the alveolated lung region.[12] Diffusional displacement, on the other hand, becomes more pronounced as particle size decreases and is the factor with the highest probability of promoting the deposition of extra-fine particles in the lung and small airways.

Settling velocity increases with the square of the particle diameter. This is why particles greater than 5 µm in size can quickly deposit after inhalation onto the oropharynx. Impaction is also an important mechanism for deposition in this area. The relationship between particle size and settling velocity means that extra-fine and sub-micron particles will take significantly longer to deposit, a primary reason why it is often thought that such particles are simply exhaled.

A typical recommended breath-hold period after dose by an inhaler is ten seconds, though this may be an overly optimistic figure when compared with the reality of clinical practice. Clearly the extent of breath hold is likely to have a marked effect on particle deposition behaviour, most especially for extra-fine particles.[10] Indeed a suggestion for increasing the deposition of extra-fine and sub-micron particles, where desirable, is to introduce longer breath hold periods, but this would require the effective training of patients and may prove problematic for some groups including the elderly and young children.

If we explore the premise that extra-fine and sub-micron particles are not simply exhaled then these particles have the potential to significantly contribute to product performance. The reduced net effect of oropharyngeal deposition (by impaction) and high pulmonary deposition in the upper respiratory region (as a result of slow settling times) could potentially counterbalance the impact of losing a certain fraction of particles in the extra-fine range on exhalation. Therefore such particles may be not only pharmacologically relevant but could even enhance dose effectiveness as a result of precise delivery to a targeted and therapeutically-relevant site. [2] Although extra-fine particles only make up a very small contribution to particle mass, they may lead to significant dosing in terms of the number of particles deposited on a receptive surface.

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Research from Bodzenta-Lukaszyk and Kokot provides some support for the suggestion that the mechanisms at play during inhalation do not result in the complete exhalation of sub-micron particles.[13] This study concluded that, although almost half the mass of the inhaled aerosol from an MDI was composed of particles less than 1 µm, only approximately 10% of the emitted dose was exhaled, as measured by scintigraphy.

Particles that are not exhaled and avoid deposition in the extrathoracic and tracheobronchial airways reach the alveolar region. Generally, it is thought that the principals of deposition within this region are sedimentation and via diffusion, which is of particular importance for those in the sub-micron range.[11] This reliance on diffusion and Brownian movement make the deposition of the extra-fine particles more pronounced in the alveolar region.

Assessing the Impact of Agglomeration

The preceding discussion assumes that inhaled extra-fine and sub-micron particles will remain discrete. However, at this size particles have a sharply increased tendency to agglomerate due to inter-particle forces which increase exponentially with decreasing particle size. That said, agglomerates are not fixed units, a primary factor in inhaled drug delivery, and can change their size and shape depending on the surrounding conditions. Larger agglomerates may break down into smaller particles, or smaller moieties may agglomerate further to form even larger particles.[14]

In the design of a DPI, particle de-agglomeration is actively promoted by inducing an inspiratory airflow that creates turbulence and aerosolisation within the device. Low-resistance devices result in high inspiratory flow rates while higher resistances induce lower air velocities. Due to the settling velocity and agglomeration tendencies of sub-micron particles, the development of inhalers with highly effective de-agglomeration mechanisms, typically those with high internal resistance, are therefore more likely to provide greater lung deposition than those with a lower internal resistance.[15,16,17] At the same time as promoting successful de-agglomeration these devices deliver the low velocity needed to offset the slow settling rates and ensure the successful administration of extra-fine particles.[18]

Accounting for the Effect of Humidity

One further factor to consider when investigating the behaviour of particles in the respiratory system is the geometric expansion of particles that may occur as a result the high humidity levels (of 99.5%). This effect means that hygroscopic particles will have different deposition patterns from analogously sized non-hygroscopic alternatives. The size increase experienced by hygroscopic particles will directly affect settling velocity and therefore the location of deposition.

In vitro it is very difficult to mimic the extreme humidity of the respiratory system due to the impact these conditions have on instrumentation. Interpretations and extrapolations therefore have to be made as to how 99.5% humidity will affect particle behaviour, with only *in vivo* investigations being able to demonstrate the impacts accurately.

INVESTIGATING & MODELLING EXTRA-FINE PARTICLE BEHAVIOUR

Experimental studies have demonstrated that factors including airway wall motion, inhalation waveform and geometric complexity all influence the deposition of aerosols in the respiratory system by affecting particle impaction and sedimentation.[19] As such, much work has been undertaken to investigate the best physical models to develop correlations of aerosol depositions that can be used to predict the doses deposited at target locations within the lung, including the alveolar dose.

In routine OINDP testing, multistage cascade impactors are the instrument of choice for measuring data related to deposition behaviour as they enable generation of an aerodynamic particle size distribution specifically for the active pharmaceutical ingredient, across an appropriate size range. These instruments are precision engineered and separate a sample via particle inertia. However, they are designed primarily to determine the consistency and quality of inhaled products and do not accurately represent the anatomical complexity of the human airways.

The metric MMAD, generated from cascade impaction testing, excludes the particles depositing in the "throat" area of the apparatus. A number of studies have shown that extra-fine drug formulations with a low MMAD give greater lung deposition compared with larger particle formulations.[20]

Physical models of the pulmonary acinus regions have been developed to investigate the deposition of pharmaceutical products further in order to predict therapeutic effect. Depending on the drug being delivered and its desired clinical effect, it can be necessary to target certain regions of the pulmonary system. For instance, for some pharmaceuticals, the alveolar region may be the target for deposition due to the need to address impaired performance in this region which is often an issue for asthma sufferers.[5,9] Conversely, for other drugs the tracheobronchial region may be the target and deposition in the alveolar region could potentially cause unwanted systemic exposure and increased side effects.[19]

Individual alveolus models consisting of a single hemispherical shell or single alveolus attached to a tube have been used to characterise general transport within the alveolar region. The complexity of such models has since been increased to include channels with multiple hemispheres attached. Other models include rectangular alveoli compartments and bifurcating networks using a honeycomb structure of attached alveoli.[19]

It has been found that the behaviour of particles varies greatly depending on the characteristics of the model used, which makes it difficult to compare the particle behaviours observed in different studies.

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Models with multiple alveoli have been found to affect the flow field that pertains and particle trajectories, while bifurcations and complex airway geometry strongly influence aerosol deposition.[21, 22]

Further, Navvab Khajeh-Hosseini-Dalasm and P Worth Longest found that alveolar deposition is dependent on deposition in the upper airways because of the impact this has on the remaining fraction of particles that enters the deep lung region.[19] Models mimicking wall motion,

which drives alveolar airflow, have shown that such movement is also an important component with unsteady state flow having a large effect on particle transport and deposition.[23] It has also been suggested that truncated acinar models can be implemented to capture total deposition and, when combined with factors such as the impact of gravity, angles and sedimentation, these enable the development of new correlations to predict aerosol deposition from an inhaler device, from the tracheobronchial airways to the alveoli.[24]

The measurement of extra-fine particle behaviour is technically challenging, regardless of the physical model. However for studies investigating the deposition of inhaled drugs at the extra-fine level, techniques that combine scintigraphy with computed tomography imaging do allow 3D assessment of the particles' regional deposition with reasonable accuracy.[13] Since research suggests that targeting the peripheral airways with smaller drug particle aerosols certainly achieves comparable and in some cases superior drug efficacy, [4,5] such studies are important for the ongoing development of inhaled products.

THE COMPLEMENTARY ROLE OF MODELLING

When *in vitro* models are used to simulate discrete aspects of pulmonary particle behaviour, the data generated from such studies is often extrapolated using some form of computer model to provide a prediction as to how the results can be applied to the whole lung. Such models are used as a means to correlate *in vitro* and *in vivo* studies better by bridging the gap between *in vivo* behaviour and the data generated by cascade impactors and other physical set-ups that fail to capture particle behaviour precisely. As alveoli are extremely small, many studies use computational fluid dynamics (CFD) alongside scaled-up *in vitro* models for the analysis of aerosol transport and deposition.[8]

This type of work is exemplified by work conducted by Khajeh-Hosseini-Dalasam and Longest assessing models used for the study of particle behaviour in the pulmonary system.[19] These researchers found that airway wall motion was important to match *in vivo* alveolar deposition data with one-dimensional (1D) models accurately. These models are used to implement analytical approximations of the various particle transport mechanisms to predict deposition at the level of individual bifurcations throughout the airways.[25] 1D models, however, only consider distance travelled in a tubular network so omit the importance of considering oscillating flow and wall motion, which are important for matching particle behaviour and alveolar deposition *in vivo*.

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CFD uses mathematical algorithms to simulate the motion of particles, fluids and gases and their interactions with surfaces and is increasingly being used in medical studies. CFD approaches have recently been developed to simulate the delivery of pharmaceutical aerosols throughout the conducting airways.[11] The spray and jet effects of the inhaler are captured in addition to the patient's inhalation profile. By evaluating a sufficient number of stochastic individual path (SIP) models, the regional deposition fractions within the lung emerge. CFD models can successfully account for bifurcation asymmetry – alveolar branches have a random orientation in the airways – and physical effects on pharmaceutical aerosols, and enable the accurate prediction of highly localised deposition factors, which is crucial when studying the behaviour of extra-fine and sub-micron particles for pharmaceutical purposes.[26,27]

These models can potentially also be used to help determine the aerosol penetration fraction that exits the bronchioles and enters the alveolar region over time. However, CFDs have not extensively been used for investigating alveolar deposition and are still in the developmental stages with respect to predicting deposition in this region. Physically relevant factors such as inhalation profiles consistent with pharmaceutical delivery still need to be taken into account.[19] However CFD models can relatively accurately model the two inhalation manoeuvers most commonly applied when actuating inhalers – "slow-and-deep" and "quick-and-deep", both of which are usually followed by a period of breath-hold. This period is often also included in CFD models to simulate particle deposition accurately.

In terms of the specific results obtained with CFD studies, one study found a significant difference in the prediction of particle deposition with both inhalation manoeuvers using a CFD alveolated model with moving walls, compared with a 1D solution.[19] The conclusion from this work was that 1D models are not ideal for accurately predicting deposition in the alveolated airway. The CFD model however demonstrated the formation of accumulations of particles – deposition 'hotspots' – and so could be used to predict regional drug delivery within the airways.

The analysis of data from CFD models suggests that excellent approximations of *in vitro* extra-fine particle behaviour can be made in terms of velocity and deposition. Further comparisons of data with *in vivo* study findings see patients undergo CT scans after the administration of radiolabelled medication to establish deposition masks of the left and right lung regions, the oropharyngeal region and the gut.[28] Studies of this nature are crucial since it is widely recognised that *in vivo* investigations are the most pharmacologically relevant. The current limitations of computer simulations and *in vitro* methods means that well designed *in vivo* studies remain key to understanding the full potential and opportunity for extra-fine drug delivery to the lungs.

LOOKING AHEAD

Extra-fine aerosols potentially offer pharmacological benefit for pulmonary delivery. Not only is there evidence to suggest that extra-fine particles may reach the deep lung, rather than be exhaled, but also that this can result in a more uniform dosing [3,4]. This can be a benefit for improved local therapeutic effect, and potentially for systemic drug delivery too.

To progress this field of study further, large scale *in vivo* studies are needed to clinically determine the path of extra-fine and sub-micron drug particles through

the pulmonary region and to validate the *in vitro* and computational investigations being conducted. This is important to progress our understanding of the extra-fine fraction already produced by existing inhaled products. The exploitation of extra-fine particle behaviour through the development of a new generation of inhalers designed to deliver nanosized particles is a separate challenge that lies well beyond current capabilities.

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