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Lactose characteristics and the generation of the aerosol

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Abstract

The delivery efficiency of dry-powder products for inhalation is dependent upon the drug formulation, the inhaler device, and the inhalation technique. Dry powder formulations are generally produced by mixing the micronised drug particles with larger carrier particles. These carrier particles are commonly lactose. The aerosol performance of a powder is highly dependent on the lactose characteristics, such as particle size distribution and shape and surface properties. Because lactose is the main component in these formulations, its selection is a crucial determinant of drug deposition into the lung, as interparticle forces may be affected by the carrier-particle properties. Therefore, the purpose of this article is to review the various grades of lactose, their production, and the methods of their characterisation. The origin of their adhesive and cohesive forces and their influence on aerosol generation are described, and the impact of the physicochemical properties of lactose on carrier-drug dispersion is discussed in detail.

Keywords: dry powder inhaler, carrier, particle interaction, surface properties, characterisation, inhalation

Abbreviations

AFM	Atomic Force Microscopy
CI	compressibility index
D(0.5)	mass median diameter
D_{ae}	aerodynamic diameter
DPI	dry-powder inhaler
DPPC	dipalmitoylphosphatidylcholine
DSC	differential scanning calorimetry
DVS	dynamic vapor sorption
ED	emitted dose
ELPI^{TM}	electrical low-pressure impactor
FPD	fine particle dose
FPF	fine particle fraction
HPMC	Hydroxypropyl methyl cellulose
HR	hausner ratio
IGC	inverse gas chromatography
LOQ	limit of quantification
MMAD	mass median aerodynamic diameter
Mg st	magnesium stearate
PSD	particle size distribution
RH	Relative humidity
RSD	relative standard deviation
SCF	supercritical fluid
SEM	Scanning Electron Microscopy
XRPD	X-ray powder diffraction

1. Introduction

Dry-powder inhalers (DPIs) are routinely used in the treatment of respiratory diseases. They are a widely accepted inhaled delivery dosage form, particularly in Europe, where they are currently used by an estimated 40% of patients to treat asthma and chronic obstructive pulmonary disease [1]. Successful drug delivery depends on the interaction between the powder formulations and the device performance to generate a suitable aerosol. To achieve deep lung penetration, drugs are often micronised to sizes between 1 and 5 μm. However, small drug particles generally have poor flow properties and are notoriously difficult to disperse due to their highly cohesive nature. They tend to adhere and remain in the DPI device during the emission process, resulting in low aerosol generation and unreliable dosing [2].

Therefore, to improve flow dispersion, a population of coarse particles (50-100 μm) is incorporated into the formulation to serve as carriers onto which the drug particles adhere during blending [3]. Because carrier particles must be inert, have a safe toxicological profile, possess a physical and chemical stability compatible with the drug substance, and be readily available and inexpensive, lactose is the most commonly selected carrier [4, 5]. In fact, for a long time lactose was considered as the excipient of choice in solid oral dosage forms. Its use spread to the inhalation field, and nowadays various inhalation grades of lactose with different physico-chemical properties are available on the market.

These lactose-containing DPI powder mixtures are often called ordered or interactive mixtures, which are easier to handle during the manufacturing processes. Moreover, usually no more than a few milligrams of a drug needs to be delivered (e.g., between 20 μg and 500 μg of corticosteroids for asthma therapy), and thus lactose provides bulk, which improves the handling, dispensing, and metering of the drug. A typical drug-to-carrier ratio is 1:67.5 [6-8].

During inhalation and aerosol generation, the drug particles are dispersed from the surface of the lactose particles by the energy of the inspired air flow that overcomes the adhesion forces between drug and carrier. The larger carrier particles impact in the upper airways, whereas the small drug particles penetrate into the lungs. However, attention must be paid to excessive adhesive forces that may prevent the elutriation of the respirable particles from the carrier surfaces, leading to upper airway deposition of the drug agglomerated on the carrier [9]. Ideally, the balance between adhesive and cohesive forces should be adjusted to a level that provides enough adhesion between drug and carrier to provide a stable formulation (homogeneous mixture with no powder segregation and good content uniformity) yet allows for easy separation during inhalation. Therefore, it has been recognised that the efficiency of a powder formulation is highly dependent on the lactose quality, lactose source, particle size and particle size distribution (PSD), fine-lactose content, and the inhalation flow rate and dispersion capacity of the device with respect to the DPI used. The surface roughness and the shape of the lactose particles have also been described as having a major influence on the aerosol generation properties [8-10]. Consequently, the selection of lactose is a crucial determinant of the overall DPI performance.

This paper provides a review of the various grades of lactose, their production and the methods of their characterisation. Moreover, the physico-chemical properties of lactose and their influence on aerosol generation are described, and the impact of the main forces of interaction on carrier-drug dispersion is discussed. In fact, the lactose carrier particles must be carefully selected and characterised because their physico-chemical characteristics profoundly affect the performance of the formulation and its aerosol generation.

2. Fine-particle evaluation, particle interactions and aerosol generation

2.1. Particle aerodynamic diameter and lung deposition

The delivery principle of inhalation aerosols is based on the generation of a population of liquid or solid particles that are carried along by the patient's inspiratory airflow and are finally deposited in the different levels of the respiratory tract based on their aerodynamic size distribution. In the inhalation field, the aerodynamic diameter (d_{ae}) is the most appropriate particle-size expression because it considers particle dynamic behaviour in airflow, which depends on the size, density and shape of particles, and describes the main deposition mechanisms (i.e., inertial impaction and gravitational sedimentation) [11, 12].

In the respiratory tract, particles larger than 5 μm, such as the lactose carrier, are mainly impacted in the upper respiratory tract (i.e., oropharynx), where the airflow shows a high velocity and changes direction drastically (e.g., in the throat). Particles between 1 and 5 μm (e.g., drug particles) are mainly deposited by sedimentation in the lower respiratory tract (i.e., bronchial tree and alveoli), where the air velocity progressively decreases. To specifically reach the alveolus tissue, the particle d_{ae} needs to be in the range of 1-3 μm [13]. Below the size of 0.5 μm, Brownian motion characterises the displacement of particles, which may be deposited by diffusion but are mostly exhaled by the expiratory airflow. In addition to these major deposition mechanisms, interception and electrostatic forces may also participate in particle deposition in the lungs [14].

To evaluate the *in vitro* aerosol performance of the formulation/device combination in delivering drug particles in the appropriate aerodynamic size range, the American and European pharmacopoeias have described methods based on inertial impaction to determine the fine particles for each inhaled drug-delivery system [15]. The aerodynamic assessment

methods of fine particles permits to determine the fine-particle dose (FPD), which represents the mass of drug particles that have a $d_{ae} < 5$ μm, which can, theoretically, be deposited in the deep lung after inhalation, and the fine-particle fraction (FPF), which is the percentage of the FPD usually related to either the nominal dose (total drug mass contained in the device) or the metered and recovered dose (total drug mass recovered in the device and in the different parts of the impinger after inhalation). The FPF can also be related to the emitted dose (ED), which represents only the drug mass exiting the device after inhalation. The ED is usually used as an indication of the ability of the powder to be fluidised by the airflow through an inhaler, while the FPD and FPF measure the capacity of the formulation to be fluidised and deagglomerated in time to release the drug from the carrier to be deposited in the appropriate level of the impinger. The mass median aerodynamic diameter (MMAD) is the diameter at which the aerodynamic particle size distribution expressed in mass is separated into two equal halves.

The pharmacopeial methods are mainly focused on the dispersion and deposition of drug particles without considering the deposition of lactose particles in the different stages of the impinger. Karhu et *al.* evaluated the pulmonary deposition of different lactose carriers in ten healthy volunteers using gamma scintigraphy [16]. Different lactose carriers were chosen with narrow and broad PSDs, which were characterised by a low or high fraction of fines, and with different mean particle sizes (20.5, 49.0 and 62.1 μm). The resulting pulmonary deposition was low, between 2.5 and 3.3% for the different lactose carriers, even for those possessing a higher fraction of fines, which seemed to remain on the carrier without being detached during inhalation [16].

2.2. Adhesive-cohesive forces

2.2.1. Interparticulate interactions

Interparticulate interactions (both cohesion, i.e., drug-drug, and adhesion, i.e., drug-carrier) are dominated by physical forces of interaction: i) van der Waals forces, which are considered to be the most substantial forces, ii) electrostatic charges, iii) capillary forces and, iv) mechanical interlocking [11]. Other interactions are determined by chemical forces, such as acid-base interaction forces and hydrogen bonding [17]. The different interparticulate interactions between the drug and lactose carrier are illustrated in Figure 1. The order of magnitude of the physical forces varies with particle size, shape, surface properties, the hardness of the adhering particle, surface roughness, contamination of the carrier particle, the intensity (and duration) of the press-on forces during mixing, and the relative humidity (RH) [18]. In the case of formulations consisting of micronised particles (e.g., drug and fine lactose) and a larger carrier, the size difference between the fine particle and the carrier allows a type of adhesion to be considered that is basically the same as that between a sphere and a flat surface. The magnitude of the adhesive force for this situation is proportional to the diameter of micronised particle and varies following the distance between micronised particle and the carrier (for van der Waals forces and electrical charges) or following the surface tension of the liquid between particles (for capillary forces) [18].

2.2.1.1. Van der Waals forces and mechanical interlocking

The dominant interaction forces between micron-sized particles in a powder are van der Waals forces of attraction [19]. The electrodynamic van der Waals forces can be understood by imagining an instantaneous picture of molecules possessing different electronic

configurations, giving them a dipolar character. This temporary situation will act on the neighbouring molecules rendering these also dipolar. As a consequence of the general attraction between dipoles, molecules will attract each other, even when they are apolar [19]. Van der Waals forces dominate gravitational forces when the separation distance between particles is sufficiently small (< 100 nm) and the particle size is sufficiently small (< 10 μm), as is the case in formulations with drug and carriers [19]. Consequently, geometrical factors, such as surface roughness, may affect the magnitude of van der Waals forces. In fact, the surface asperities may limit the closeness of two particles to each other and increase the interparticulate distance, which thereby limits the van der Waals attraction to almost zero when the asperities are of the order of 1 μm. On the other hand, the intimate contact area could substantially increase the van der Waals forces of attraction and may cause mechanical interlocking when protuberances fit into cavities.

2.2.1.2. Capillary forces

Capillary forces or "meniscus forces" arise due to the formation of a liquid concave-shaped meniscus (liquid bridge) around the contact area of two neighbouring particles [20]. When two particles enter into contact with each other, a narrow slit is created around the contact surface. If the two solid surfaces are lyophilic and the gap is sufficiently close, some vapour will condense from the surrounding vapour and form a meniscus; this phenomenon is called "capillary condensation" and is quantified by Kelvin's equation [20]. Capillary condensation happens even if the partial pressure of the liquid is less than the saturation vapour pressure. The attractive force caused by the concave-shaped meniscus is due to the surface tension of the liquid around the periphery of the meniscus, which pulls the particles together. In addition, the pressure inside the meniscus is reduced compared to the outer pressure by the negative

Laplace vapour pressure acting for curved liquid surfaces. This pressure difference acts over the cross-sectional area of the meniscus and attracts the particles towards each other [20]. Capillary forces have been reported to vary from a few nanoNewtons (nN) to a few hundred nN for organic drug crystals over a range of humidities [21]. The magnitude of these forces, involved in adhesion and cohesion, varies according to drug or carrier physico-chemical properties, such as shape and size, as well as the roughness and chemical properties of the surface, but especially according to the environmental RH [20].

2.2.1.3. Electrostatic charges

Electrostatic charges occur when two dissimilar surfaces are brought into contact and then separated, resulting in oppositely charged surfaces due to a charge transfer between a donor and an acceptor. When the contact is made by a short collision or by intense friction, the resulting charging phenomenon is called “triboelectrification” [22, 23]. Contact charging is classified into three categories according to the contacting materials: metal-metal, metal-insulator, and insulator-insulator contacts [22, 23]. In the pharmaceutical field, most drugs and excipients are organic crystals, which present high resistivity and poor conductivity and therefore behave as insulators under ambient conditions. The contact surface may be metal (the mixer vessel) or insulating materials (the device’s plastic components as well as the excipient or drug particles during mixing and fluidisation).

Triboelectrification, arising from manufacturing processes (mixing, handling, and filling), is considered to be a nuisance because it limits powder flowability during the industrial process by increasing the adhesive-cohesive forces between powder particles, which decrease aerosol performance. The materials of the mixer vessel (stainless steel) and the device constituents (polypropylene and acetal) influence adhesion as well as the magnitude and polarity of the

charges acquired by the powders as a function of the nature of the powder [24]. Furthermore, the addition of a fine-lactose excipient (<10 μm) to coarse lactose decreases the magnitude of triboelectrification during mixing [25]. The increase in amorphous content and the use of a carrier with a wide PSD affects the polarity and the magnitude of the lactose triboelectric charges [26, 27].

Moreover, triboelectrification also occurs at the moment of the fluidisation of the bed powder through the inhaler by the inspiratory airflow. The resulting electrostatic charges are involved in particle deposition in the lungs and can be used to enhance lung deposition [28]. Chow et *al*. evaluated the triboelectrification of a inhalation-grade coarse lactose (Inhalac® 230) at different stages of manufacturing and aerosolisation [29]. The initial negative charges were low and decreased with an increase in the RH. During the mixing step, the triboelectric charges increased, and no dissipation was observed after 30 min of holding time. Handling powder in a gelatin capsule and tapping powder simulated transport-induced triboelectrification. The sign of the charge of Inhalac® 230 did not change during these different processes. During the aerosolisation of the powders, the magnitude of charge was much higher than those of both the initial and the capsulated bulk powder. Moreover, the charge polarity of the lactose carrier changed due to contact of the lactose with the plastic inhaler material. The airflow and the RH have a linear correlation with the production of electrostatic charges [29].

In the case of the drug-lactose carrier mixture, the net charge on the drug particles could arise from the drug separation from the carrier particles, from the surfaces of the dosage form (e.g., capsule), and/or from the inhaler. The lactose grade (sieved or milled), inhaler device and capsule material have a strong effect on both the magnitude and the polarity of electrostatic charges generated by the aerosolisation of dry powders [30, 31]. The design and deaggregation mechanisms of the inhaler have an impact on the triboelectrification of drug

and lactose carrier particles [27, 32]. In addition, external factors such as storage RH affect the magnitude of electrostatic charges of drug particles during inhalation and therefore have an impact on the *in vitro* aerodynamic performance (see Section 2.2.3) [33].

Static (i.e., within the powder bed) and dynamic (i.e., during inhalation) electrifications of dry powder for inhalation are important factors that must be taken into consideration and must be better understood to improve the use of DPIs. It is important to evaluate the triboelectrification of the dry-powder formulation arising from manufacturing processes, which could influence the adhesion forces affecting flow properties and *in vitro* aerodynamic performance, and that from powder aerosolisation, which could increase particle deposition in the lung.

2.2.2. Surface properties

The adhesion of drug particles to the carrier is also a surface-interaction phenomenon. The magnitude of the adhesion forces, which influence the aerosol performance, depends on the contact area between the drug and carrier, the particle geometry of the carrier and the surface energy of the contiguous surfaces. Different strategies based on the modification of the surface properties of the drug and carrier particles have been used to improve the aerosol performance. Some authors increased the roughness of the drug particles to decrease the contact area between the drug particles and the carrier, which decreases the van der Waals forces that are mostly involved in the adhesion forces [34-36]. Other strategies have modulated the adhesion forces between the drug and carrier by modifying the crystal habit of the drug to change the molecular orientation of the crystal faces on the surface. However, no direct effect was observed on the rank order of the cohesion-adhesion force balance values obtained with the different carriers that have shown a correlation with the aerosol

performance [37]. In the case of the lactose carrier, the reduction of the roughness was initially the most frequently investigated strategy because it leads to a reduction of the surface area and consequently a decrease in the van der Waals adhesion forces (see Section 3.1.3).

The lactose carrier is also characterised by surface heterogeneity, implying a non-homogeneous energy distribution and morphology on the surface, which vary according to the industrial processing (see Section 3.1). The non-homogeneous energetic distribution is characterised by the presence of active sites of enhanced energy at the carrier surface (Fig. 2.A). Generally, active sites can occur in morphological regions (peaks and troughs), amorphous regions (from milling or spray-drying processes), in the presence of impurities (such as proteins and some fat present on lactose carrier extracted from milk) and in specific polar/nonpolar regions (dispersive, acid/base energetics), which decrease drug detachment during inhalation. Thielmann et *al.* evaluated the surface energy distribution of untreated, milled, and recrystallised (after having milled) 63-90 μm sieved lactose monohydrate [38]. The PSDs were similar but both the amorphous content and the roughness morphology were different (milled > untreated > recrystallised). A similar dispersive surface energy (~42 mJ/m^2) was found for the untreated and recrystallised lactose, but the energy distribution varied greatly. This variation was attributed to the samples' anomeric compositions or to impurities. The milled lactose presented a shift in the energy distribution of both dispersive and specific (acid/base) components to higher values (~53 mJ/m^2) compared to the other two samples, which was attributed to the amorphous content [38].

Consequently, the active sites of enhanced energy are preferentially occupied by small particles with a higher interaction compared to sites with low energy [10, 39]. This factor could be determining for low-drug-dose formulations because, below the critical adhesion limit, the drug particles saturate the active sites and the aerosol performance decreases. Above the critical adhesion limit at which the saturation of active sites occurs, the drug particles also

occupy non-active sites, which are characterised by lower adhesion forces and therefore by better aerosol performance. For example, the relationship between the drug/lactose ratio and the aerosolisation performance of conventional carrier-based formulations containing salbutamol sulphate has been investigated by studying a dose range of 10–450 μg of drug in a 50 mg α-lactose monohydrate (63-90 μm) carrier formulation [40]. No statistically significant difference in FPD was observed for drug levels between approximately 10 μg and 135 μg. However, increasing the dose from 135 μg to 450 μg resulted in a statistically significant increase in FPD. This observation may be attributed to the occupation of active carrier sites, which are characterised by a strong binding interaction, by drug particles at low drug concentrations. It is assumed that sites with high energy on the carrier surface would be preferentially occupied compared to sites with lower energy as a consequence of a combination of increased contact area, high surface free energy and simple geometric constraints. For example, micron-sized drug particles may accumulate in a recess in the surface of a large lactose carrier particle (Fig.2.B) [40].

Strategies using a mechanofusion process with additives to homogenise the distribution of adhesive forces over the carrier surface or fine particles to modulate the adhesion of drug particles to active sites to improve aerosol performance are discussed in Sections 3.1.3 and 4.1.2, respectively.

2.2.3. External factor: relative humidity

The RH of ambient air in contact with DPIs plays an important role in the magnitude of the adhesion forces dominating the dynamic behaviour of dry powders. RH may have a versatile function on the electrostatic charges. The increase in ambient RH promotes the formation of water layers around particles, which increases the surface conductivity. In static conditions

(e.g., storage), the increase in conductivity allows the relaxation and dissipation of charges accumulated on powder particles, which results in a decrease in electrostatic charges and therefore in an increase of aerosol performance [29, 33]. It should be kept in mind that the capillary forces dominate the adhesion force above a certain level of RH and decrease aerosol performance [33]. Under dynamic conditions (e.g., aerolisation), the increase in conductivity could promote charge transfer and the generation of additional electrostatic charges by the ionic dissociation of water [29].

Moreover, the water layers adsorbed on the particle surface may progressively modify the surface topography and, as a consequence, the adhesion forces [41]. Das et *al.* demonstrated that, at higher levels of relative humidity, the total surface energy increased with an increasing polar energy component for the micronised drug (salbutamol xinafoate) and coarse and micronised lactose, which all possess polar and non-polar groups in their structures [42]. These variations are reversible in the short term but could be irreversible after long-term storage due to structural changes (solid bridge) in the agglomerates that entrap the drug and therefore decrease the aerosol performance [43]. In a blend containing coarse and fine lactose, high RH during long storage makes the surface of the coarse particles smoother by dissolving the fine particles present on the surface. The agglomerates of fine particles are progressively linked by solid bridges due to the dissolution and recrystallisation of lactose in the liquid bridge by capillary condensation [44].

Amorphous regions resulting from production processes (e.g., milling) are particularly sensitive to variations in temperature and humidity. At high RH, these regions recrystallise and may fuse with a contiguous surface, modifying their surface properties [45]. In addition, hygroscopic drugs also present a greater risk of physical and chemical instability at high RH [46].

2.3. Mechanisms of aerosol generation

The efficacy of DPIs depends on the extent to which the primary drug particles in the formulation can be dispersed into a suitable aerosol during inhalation. Therefore, one of the main challenges in the inhalation field is to achieve delivery of the highest dose fraction of drug to the lung with high reproducibility. Inadequate drug-carrier separation is one of the main causes of great variability and low deposition results [10]. Consequently, the aim in DPI development is to produce drugs with an appropriate MMAD and to select carriers with suitable characteristics to generate an efficient aerosol and achieve deep drug deposition in the lungs.

Specifically, aerosol generation requires the powder to overcome interparticulate forces (see Section 2.2.1) binding particles in bulk powder and to become entrained as single particles in the inhalatory airstream. Despite active research in the field of DPI development, the phenomenon of deaggregation remains complex. The whole aerosolisation process can be roughly categorised into four phases, starting from a static powder bed to dilation, fluidisation, and finally drug resuspension [31], which are performed by the main deagglomeration forces, including turbulent, inertial and impact stress, before drug deposition in the respiratory tract (Fig. 3). These phases are considered to be concurrent rather than stepwise. When the airflow entrains through a DPI device, it transfers the kinetic energy of the continuous bombardment of air molecules into the powder bed for powder entrainment and deaggregation. The initial mechanism of powder-bed break-up is shear fluidisation, with the particles entrained into the flow layer-by-layer, producing a slow particle source. Following this process, the jet flow penetrates the powder bed and aerates the entire bed from the centre, producing a fast particle source. Two lactose powders containing different proportions of fines (6% and 16%) exhibited a fracture mechanism during fluidisation that resulted in large agglomerates breaking off from the powder bed as it cracked along lines of

weakness [47]. Of course, the porosity of the powder, which is the ratio of the volume of all voids in a powder bed to the total volume, provides a measure of the ease with which a flow can move through a stationary powder bed: the higher the porosity, the more void space is available for the air to flow through [47]. A direct correlation of particulate interaction with the aerosol performance is difficult because of the heterogeneous nature of both the drug and carrier surfaces (see Section 2.2.2) and assumptions with respect to force measurement.

The aerosol generation and consequently the FPF obtained during inhalation from a DPI is the result of a competition between the interaction forces within the powder and the separation forces derived from the inspiratory airflow through the inhaler. Separation forces for particles adhering to the surface of a carrier particle, which can be derived directly from the kinetic energy of the inspiratory airstream, are drag and lift forces, shear and friction forces and inertial forces [48]. For micronised particles attached to a carrier crystal, drag and lift forces are not the most effective types of separation forces because they are widely proportional to the first power of the particle diameter and act only when a velocity difference between the air and the particle exists. This difference occurs when the carrier particle impacts on a surface and suddenly reduces its velocity, during sudden changes in the velocity of a carrier particle as it is passed through a turbulent flow, and during mechanical vibration [49]. Inertial forces are the most effective type of separation force for drugs attached to carrier particles [50]. They include vibration, centrifugal and collision forces. If the cohesion forces (drug-drug interactions) in drug-lactose agglomerates are stronger than the adhesion forces (drug-carrier interactions), the agglomerates composed of micronised drug may be released as a whole, requiring a much lower velocity than the detachment of a single particle because of the much higher inertia for the agglomerate [51]. The impaction force exerted on a drug particle attached to a carrier is proportional to the rate of change of the carrier-particle velocity and the drug-particle mass. It has been shown that a slight increase in the mean drug particle

diameter may already cause a substantial increase of the percent of drug detached from the carrier [52].

In fact, for DPIs, the dose reaching the lungs is dependent on four interrelated factors [1]: 1) the properties of the drug formulation, particularly powder flow, particle size and drug-carrier interactions, 2) the performance of the inhaler device, including aerosol generation and delivery, 3) correct inhalation technique for deposition in the lung, and 4) the inspiratory flow rate. Therefore, attention must be paid to all of these parameters. Moreover, based on the device construction (DPI geometry, dimension, and dosing method) and the inspiratory flow of the patient, the mechanism of fluidisation and deaggregation may vary greatly.

2.3.1 Drug formulation

For dry-powder dispersion, attractive forces between primary particles of agglomerates have to be overcome. Thus, deagglomeration only occurs when the stress is larger than the stability of the agglomerate due to the adhesive forces. According to Rumpf's theory, particle separation occurs when the adhesion forces are supplanted by the applied removal force [49]. Therefore, stress has to be introduced in smaller units and accumulated over time and distance to effect an ultimate total disintegration. In the case of an uncompleted disintegration, agglomerates still remain. An incomplete deagglomeration is to be expected for dispersion over a short time, in which the agglomerates leave the process before the complete cascade of deagglomeration is finished, and/or if the stress from a deagglomeration mechanism is not sufficient to reach the minimum value that is required to separate the primary particles from the agglomerate. From a practical point of view, it can be expected that cohesion between primary particles is not homogeneously distributed in agglomerates. In this case, agglomerates

are first divided into parts along areas of lesser cohesion so that smaller and relatively stable agglomerates ultimately remain. Therefore, the question would be how much stress is required to separate the remaining small agglomerates of higher stability [48].

Consequently, there is a need for new formulations and particle engineering that significantly decrease the particle adhesive forces and improve the aerosol generation of the formulations such that the required flows are lower for these powders and the PSD is not sensitive to flow rate. For example, it has been recognised that the carrier surface properties are very relevant to the drug-to-carrier interaction. Therefore, techniques including particle smoothing, passivation of the active sites and, the addition of force-controlled agents and formulations of interactive mixtures are further developed in Sections 3.1.3 and 4, respectively.

2.3.2. Inspiratory airflow

Because DPIs are activated by the patient's inspiratory airflow, the effectiveness of the aerosol generation is susceptible to the vagaries of age, gender, disease, and the breathing cycle of the device user [3]. One of the most important disadvantages includes the fact that DPIs require moderate inspiratory effort to draw the formulation from the device, and some patients are not capable of such effort. The possibility of achieving particle deaggregation in the mouth and throat for particles of a diameter of less than 10 μm is almost non-existent at flow rates of 30 l/min or less. At a flow rate of 60 l/min, the break-up of 10 μm diameter or smaller agglomerated particles can potentially occur, starting in the oropharyngeal cavity. If an agglomerate is not able to break apart in a hundredth-of-a-second time frame (the time scale for a particle to traverse an axial distance of 1 or 2 cm at flow rates exceeding 60 l/min), it will, due to its large aggregates size, possess a very high probability of depositing before it enters the trachea [53]. Because the velocity distribution of air within the lung is determined

by the tidal volume and breathing frequency parameters, the drug mass delivered to the alveoli can also be enhanced by increasing the inspiratory volume [54]. For a constant flow rate, as the inspiratory volume increases, the time of breathing increases, therefore enhancing the time for particle deagglomeration. An inspiratory volume of 3 l with an inhalation rate of 60 l/min resulted in the highest deposition in the pulmonary region of the lung [55].

Moreover, it has been demonstrated that drug deposition deep in the lung from DPI formulations is determined not only by the peak flow rate but also by the flow increase rate. It has been found that a high peak flow rate does not necessarily guarantee a high aerosol deposition if the initial flow-increase rate was insufficiently high because the drug formulation may result in incomplete dispersion, an increase of particle size and ultimately lower deposition in the deep lung [56, 57]. The flow rate and inspired volume through the DPI is determined by the resistance of the device, the lung volume of the patient, and the force that can be generated by the patient.

2.3.3. Dry-powder inhalation device

DPIs are subject to strict pharmaceutical and manufacturing standards by regulatory bodies, the most challenging of which is the demonstration of device reliability in terms of delivered dose uniformity and delivered dose deposition [58].

At present, the principal forces leading to powder deagglomeration in inhalers remain unclear. The deagglomeration of drug particles to form a fine respirable aerosol cloud is thought to be achieved by three major mechanisms within the device: particle interaction with shear flow and turbulence, particle-device impaction, and particle-particle impaction [59]. As turbulence increases, the deaggregating force increases [60]. Therefore, the design of DPIs is developed in such a way that the device should induce sufficient turbulence and particle–particle

collisions to detach drug particles from the carrier surface and achieve efficient aerosol generation. The effect of the powder interaction with the device during powder dispersion has generally been poorly understood. Recently, computational fluid dynamics has enhanced the understanding of the impact of inhaler design on powder dispersion and deposition and has demonstrated that small variations in device design can produce significant variations in performance [59, 61]. For example, increasing the voidage of the grid of Aerolizer® reduces the deagglomeration potential of the flow field generated in the device due to fewer particle-grid impactions [59]. The majority of DPIs are composed of short tubes and complex geometries through which an airflow passes that consists of a turbulent core surrounded by a laminar envelope [62]. The two factors, patient-supplied inspiratory effort and inhaler-supplied flow resistance, together yield a flow rate allowing the aerosol generation of the powder and drug deposition in the lung. The high-specific-resistance devices generate high turbulence, in general, leading to higher aerosol generation allowing higher drug dispersibility and FPFs than for low-resistance devices [63]. However, devices with higher resistance need a higher inspiratory force from patients to achieve the desired air flow. Therefore, a balance between resistance and turbulence is necessary to achieve the required aerosol generation for a desired therapeutic effect from DPI formulations.

While most DPIs are breath-activated, relying on inhalation for aerosol generation, several power-assisted devices (pneumatic, impact force, and vibratory) [64, 65] have been developed or are currently under development. It has been suggested that if shear force and turbulence could be standardised using a dispersion mechanism that is independent of the patient's breath, high delivery efficiency and reproducibility might be achieved.

In conclusion, novel particle engineering and DPI technologies have emerged, but efficiency and reproducibility in pulmonary drug delivery in an airflow-independent manner still represents a great challenge. Consequently, a balance between the design of an inhaler device, drug formulation, and the inspiratory flow rate of the patient is required [63, 66]. Nowadays, ways to improve the efficiency of aerosol generation and drug delivery to the lungs are developed by changing formulation strategy, drug- and carrier-particle engineering, and designing new devices. It is often the case that the drug formulation and inhaler device need to be optimised together to ensure reliable and effective drug delivery. Therefore, the inhaler-drug combination is generally considered as a single medication whose *in vitro* performance and *in vivo* efficacy must be demonstrated [67].

3. Production and characterisation methods of inhalation-grade lactose

3.1. Production methods of various grades of lactose

Lactose is a natural disaccharide consisting of galactose and glucose and is present in the milk of most mammals. Commercially, lactose is produced from the whey (residual liquid from the milk following cheese and casein production) of cows' milk [68].

Lactose can be obtained in either of two basic isomeric forms, namely α- and β-lactose, or in an amorphous form. α-lactose exists both in monohydrate and in anhydrous forms, the former being the most thermodynamically stable form. α-lactose monohydrate is prepared by crystallisation from supersaturated solutions below 93.5°C. Its crystalline shape can be a prism, a pyramidal or a tomahawk and is dependent on the precipitation and crystallisation methods. Anhydrous lactose (typically containing 70-80% anhydrous β-lactose and 20-30%

anhydrous α-lactose) is most often produced by roller drying a lactose solution above 93.5°C. Next, both resulting products are milled to decrease particle size and sieved to select an appropriate PSD. Spray-dried lactose is obtained by spray-drying a suspension of α-lactose monohydrate crystals in water, saturated with lactose. Approximately 10-20% of the total amount of lactose is in solution, and the remaining 80-90% is present in the crystalline form [68].

Generally, inhalation-grade lactose is characterised by stricter control of the endotoxin level and more specific restriction of the PSD than that of other grades. Moreover, a smaller particle size is often more preferable than for oral formulations. As can be seen, in Table 1, there is a wide variety of lactose with different physico-chemical properties that could be used in DPI formulations. Because the particle size, PSD and shape are important parameters for aerosol generation (see Section 2.3), inhalation grades lactose between < 5 μm and >100 μm and with various shapes can be purchased. Lactose can be either processed by milling, sieving or spray-drying, leading to different surface properties (see Section 3.1). Of particular importance, micronised lactose can be produced and added to formulations to enhance drug-particle dispersion (see Section 4.1.2.1). Besides lactose available on the market, it is interesting to note that there is active research into modifying the surface characteristics of lactose by "particle engineering" (see Section 3.1.3). Nevertheless, most of these lactose particles are characterised by a heterogeneous surface, which implies a non-homogeneous energy distribution and morphology on the surface. This non-reproducible energy distribution and morphology after manufacturing implies inter/intra-batch and supplier variation, which could influence the aerosol generation and performance of carrier-based DPI formulations [69]. Therefore, specific monographs for inhalation lactose, both anhydrous and monohydrate and including further functionality characterisation, should be introduced to limit these variations and guarantee reproducible performance of DPI products [69].

The manufacturing of DPI carrier-based powders generally includes various steps such as the production of drug and carrier particles in a suitable size range (by sieving, milling, spray-drying, etc), mixing the various components in appropriate blending conditions with optimised parameters and, if necessary, the modification of the surface properties of the particles to enhance aerosol performance.

3.1.1. Particle-size reduction

3.1.1.1 Milling

Micronisation techniques, such as ball milling and fluid-jet milling, are well established, well validated and widely used to manufacture dry powders for inhalation [11]. In fluid-jet milling, high-energy particle comminution occurs by particle-particle and particle-wall collisions under the influence of opposing high-velocity jets of compressed gases. Depending on the pressure and powder feed rate, small particles with narrow size distributions and diameters down to 5 μm can be produced [11]. In a ball mill, a decrease in median diameter from 115 ± 1 μm for the untreated lactose monohydrate to 63 ± 1 μm for milled lactose monohydrate samples has been observed. Moreover, analysis of the fines concentration with respect to mill time has indicated a significant increase from 4.4% to 18.0% for between 0 and 60 min of mill time. In fact, it has been suggested that cleavage planes, commonly found in crystals, fracture into many fine particles [70]. In general, milled lactose has a significant effect on the FPF of drugs. These effects are predominantly attributed to the fines content, showing a strong correlation between increased fines and FPF (see Section 4.1.2.1) [70]. However, increased particle aggregation may occur following particle-size reduction. As a consequence, energy may be expended in breaking up the aggregates instead of the particles, ceasing particle-size reduction. In addition, as the particles become smaller and more numerous, friction

diminishes and the sample may behave as a semi-solid. Larger particles may arch and protect smaller particles from impact, whilst smaller particles coat the grinding medium and cushion the larger particles from impact. This protection may prevent further particle-size reduction [70].

Size reduction occurs as a result of three actions: compression, shear force and impact. These three actions generate stresses on particles that, when exceeding a certain limit, will cause the permanent deformation and/or fracture of the particle [71]. In fact, the material undergoes many impact events before a significant quantity of the required particle size fraction is achieved and separated from the larger particles by inertial impaction, which alters the surface and solid-state properties. Intense milling can cause unwanted change in the physico-chemical properties of the material, e.g., creation of amorphous regions at the surface, which can affect hygroscopicity and stability, electrostatic charging and cohesivity [72]. It has been demonstrated that an increase in mill time results in an exponential increase in amorphous content [70]. In addition, the micronisation process leads to small, irregularly shaped flat lactose particles, and extensive flat surfaces promote large contact areas, resulting in increased adhesion between the particles and therefore poor flow properties [2]. Moreover, this process provides only a limited opportunity for control over important particle characteristics such as size distribution, shape and morphology.

3.1.1.2. Spray-drying

Spray-drying is a one-step process that converts a liquid feed to dried particles. The feed can be a solution, a coarse or fine suspension or a colloidal dispersion, which is first atomised to a spray form that is immediately put into thermal contact with a hot gas, resulting in the rapid evaporation of the droplets to form dried solid particles [73]. The important drying variables

are inlet and outlet temperatures, the drying-gas medium, gas humidity, gas flow rate and residence time, which all affect the final size, shape, density, crystallinity and residual solvent content of the particles. To avoid agglomeration in powders, the humidity of the gas medium must be sufficiently low, especially for hygroscopic materials. In the case of an organic feed, the vapour must be removed to reduce the residual solvent to pharmaceutically acceptable levels [73].

One of the principal purposes of aerosolising spray-dried powders is to achieve particle diameters of several micrometers with a narrow PSD due to the homogeneous droplet size distribution during atomisation and the presence of a cyclone separator for particle collection. During atomisation, the physical properties, such as viscosity, surface tension and liquid-feed density, are likely to influence the break-up of the liquid and thus the droplet size distribution of the spray. Moreover, it has been demonstrated that the nozzle orifice diameter and airflow control the droplet size during atomisation [72]. Increasing the droplet size increases the particle size, but the effect is also influenced by the feed concentration. Lactose particles obtained from solutions of a low concentration (1% w/w) were smaller than those from higher concentrations (5–20% w/w). Moreover, spray-dried lactose solutions form hollow particles, and the shell thickness of the particles seems to increase with increasing feed concentration. In view of these observations, it is likely that the droplet size and feed concentration as well as the choice of drying conditions, solvents, and solutes control not only the size but also the density of spray-dried particles such as lactose [72].

It is also important to note that spray-dried particles from solutions are mostly amorphous, but suspensions can be processed to maintain the crystalline state of the excipient or drug. For example, the crystallinity of spray-dried lactose varies from 0% to 100% depending on the ethanol-to-water ratio in the feed solution. Because lactose is practically insoluble in ethanol, crystalline lactose suspension spray-dried from pure ethanol is 100% crystalline. Lactose

solution spray-dried from pure water is 100% amorphous, and the particles are spherical and small (<10 μm). Moreover, the feed solution substantially affects the ratio of surface water to hydrate water because the content of surface water increases and hydrate water decreases, while the crystallinity of spray-dried lactose decreases [74].

It is important to note that commercial lactose (SuperTab® SD, DMV-Fonterra) for direct compression is produced by spray-drying and has been developed to ensure excellent flow and compactibility of the powder mix. The particles are more spherical than those produced by other techniques, present a narrow size distribution and contain crystals of α-lactose monohydrate and amorphous lactose. Moreover, in the Respitose® customised grade (DMV-Fonterra), commercial inhalation grade lactose may also be produced by spray-drying on demand. Nevertheless, this technique is rarely used to manufacture lactose for inhalation. In fact, the battery of controllable parameters offered by spray-drying is a great advantage over milling to produce complex engineered structures, such as porous particles and nano-aggregates, or surface-modified, coated or encapsulated materials [5].

3.1.1.3. Supercritical fluid

Supercritical fluids (SCF) are defined as compressed gases or liquids above their critical pressures and temperatures and possess several fundamental advantages as solvents or non-solvents for pharmaceutical manufacturing. Carbon dioxide, because of its accessible critical point at 31°C and 74 bars and its low cost and non-toxicity, is the most widely used solvent in many SCF processes [75]. Fine drug particles produced via SCF precipitation are less charged than those produced mechanically, which allows them to flow more freely and be more easily dispersed following a discharge from a DPI. Moreover, SCF processes permit the production

of respirable drug particles that are intrinsically more uniform in terms of crystallinity, morphology, PSD and shape than those produced via jet-milling [76].

The processability of lactose via SCF drying has recently been investigated [77-79]. Aqueous solutions were sprayed into a pressurised carbon-dioxide/ethanol mixture flowing co-currently through a coaxial two-fluid nozzle. The powder characteristics appear to be influenced by the supersaturation level reached during the SCF-drying process and by the properties of the sugar species, such as water solubility and glass-transition temperature, or the solution viscosities. The resultant lactose particles were spherical with a relatively smooth surface. The powder was free-flowing and remained amorphous during a three-month stability study [78]. Lactose has also been successfully precipitated using CO_2 as an antisolvent modified with 20% of ethanol at 20°C and 155 bar by an aerosol solvent extraction system. Lactose was shown to precipitate as thin plates smaller than 20 μm, and the powder was semi-crystalline [79]. It is important to note that high concentrations of lactose in the sprayed solutions might increase the droplet viscosity, resulting in low mass-transfer rates of the antisolvent into the droplets with correspondingly less efficient water extraction and higher coalescence and agglomeration [79].

Despite its potential, SCF is still an emerging technology that is not often used in DPI products. Concerns over the potential denaturing effects of the solvents/antisolvents used in this process is a notable drawback to its use.

3.1.2. Particle-size separation

As seen earlier, the particle size of the drug and the carrier is of particular importance in the aerosol performance of a DPI formulation. Moreover, the PSD of inhalation-grade lactose

tends to be narrower than other grades used in oral forms. Therefore, there is a need for methods allowing powders to be classified into separate particle-size ranges.

3.1.2.1. Sieving

Dry sieving processes, techniques based on mechanical disturbances of the powder bed by agitation, brushing or centrifugal methods produce a lactose grade that is not specifically used for inhalation. In agitation methods, size separation is achieved by electrically induced oscillation, mechanically induced vibration of the sieve meshes or gyration [80]. The output from gyratory sieves is often considerably greater than that obtained from the two other methods because it allows a change in the orientation of the particles and therefore an increase in potential to pass through a given sieve aperture. In the brushing method, a brush is used to reorient particles on the surface of a sieve and prevent apertures from becoming blocked. In the centrifugal method, particles are thrown outwards onto a vertical cylindrical sieve under the action of a high-speed rotor inside the cylinder. The current of air created by the rotor movement also assists in sieving, especially when very fine powders are being processed [81].

Another method based on sieving and used to produce inhalation-grade lactose is the air-jet sieving technique, in which a vacuum generates a strong jet of air that disperses the particles on the sieve through a slotted nozzle [82]. Material that is smaller than the sieve mesh size is transported by the back flow of the air to the cyclone or vacuum cleaner. The air jet helps to de-agglomerate the particles and constantly purges the sieve mesh. This process leads to short sieving times and ensures that even micronised materials with strong cohesive forces can be sieved successfully [81].

3.1.2.2. Sedimentation and elutriation

In sedimentation and elutriation methods, the separation of particles occurs by dispersion in air because lactose is soluble in water, and this method is known as mechanical air classification [81]. The centrifugal method allows the separation of finer particles than can be achieved using gravitational elutriation. Particles in an air suspension are fed into a rotating hollow torus at high speed, tangential to the outer wall. Coarse particles move outwards to the walls against the inwardly spiralling air flow, which leaves the elutriator in the centre. The desired particle-size fraction can be separated by selecting the appropriate airflow rate and rotor speed, allowing the production of inhalation grade lactose [81].

3.1.2.3. Cyclone

In this system, particles in air are often introduced tangentially into the cylindrical upper section of the cyclone, where the relatively high air velocity produces a vortex that throws solid particles out on to the walls of the cyclone. Coarser particles separate from the air stream and fall out of the cyclone through the dust outlet, whereas finer particles remain entrained in the air stream and leave the cyclone through the vortex. A series of cyclones having different flow rates and/or dimensions can be used to separate a powder into different particle-size ranges [81].

3.1.3. Carrier-particle surface modification

Attention must be paid to the balance of the adhesive and cohesive forces between the carrier and the drug when adding a coarse material to improve the flowability of the powder. For example, inclusion of a lactose carrier material (Inhalac 120) significantly improves the ED of

salmeterol xinafoate from 71.3 ± 3% for the drug alone to 81.8 ± 2.5%. Nevertheless, the FPF of salmeterol xinafoate from this binary mixture significantly decreases and is less than half of that of the formulation employing the drug only. Thus, the fine-particle generation of the drug from the carrier particles into the air stream was significantly less than that from aerosolising salmeterol xinafoate alone [83]. Inadequate drug/carrier separation is one of the main explanations for the low deposition efficiency encountered with DPIs [10]. The current methods for overcoming such issues include the production of new lactose carriers with controlled shape and roughness and "filling" the potential active sites by increasing the fine-particle content of the drug or excipient on the carrier surface or pacifying the effects of active sites by surface treatment and/or the addition of force-control agents [39].

Given that the number of carriers allowed in pulmonary drug delivery to improve the aerosol behaviour of formulations is very restricted, superior delivery efficiency may be achieved by developing optimised particulate formulations [5]. This alternative strategy, which is synonymous with the controlled production of carrier and/or drug particles of optimised size, morphology and structure, is broadly referred to as "particle engineering" [29]. Nowadays, this process is most commonly used for improving lactose and drug characteristics for inhalation to enhance the aerosol generation of the powder. Table 2 shows the main techniques of surface modification of lactose carrier with their improvements and potential limitations. The main goal of particle engineering is to incorporate desirable attributes, such as narrow PSD, improved dispersibility, enhanced stability, optimised bioavailability, sustained release and/or precise targeting [84], into particles while considering the specifics of the inhaler design and drug-delivery requirements.

A strategy consisting of homogenising several factors, such as particle size, shape and surface morphology, to increase the aerosol performance was performed by Zeng *et al.* [85]. After the recrystallisation of lactose from carbopol gels, the controlled lactose carriers had a narrower

PSD, more regular shape and smoother surface than the conventional lactose carriers obtained by recrystallisation under constant stirring [85]. The DPI based on salbutamol sulphate particles presented a better dispersion and deagglomeration from the controlled lactose carrier at different flow rates (28.3, 60.0 and 96.0 l/min) than from the control groups [85].

To produce a lactose carrier with controlled roughness to obtain an ideal morphology, Young *et al.* developed a lactose composite made with a subunit of spray-dried lactose of either 2, 6 or 10 μm that yields a homogeneous roughness surface with a macroscopic shape and a PSD that is relatively similar to conventional lactose carrier [86]. It was shown that a linear relationship exists between the roughness and the FPF and that an inverse linear relationship relates the median adhesion force to the FPF. In this case, the surface roughness (regular lactose < 10 μm < 6 μm < 2 μm lactose composite) decreased the contact area between drug and the carrier, which resulted in lower adhesion forces (2 μm < 6 μm < 10 μm lactose composite < regular lactose) and higher aerosol performance. Moreover, the variation seen in the distribution of adhesion forces was reflected in the variation in the FPF [86].

Moreover, because the carrier surface is known to be of particular importance, a mechanofusion process has been developed, which consists of homogenising the particle surface or dispersing a minimally melting or a very fine solid material around the carrier particles using intensive mechanical processing [87, 88]. This technique can modify the surface energy of lactose carrier particles without drastically changing the particle size and can make the particle shape round to lessen the differences and variations across the particle surface. Through the mechanofusion process, compression and shearing energy is added to the lactose particles when they pass through the narrow gap between the rotor and press head of the mechanofusion apparatus [87, 88]. These new lactose grades with modified surface properties could find their way into the market in the near future as a novel excipient because

there is no need for expensive and time-consuming toxicological studies to be approved by the authorities.

Moreover, force controlled agents such as magnesium stearate (Mg st), which is well known as a lubricant and utilised to modify DPI formulation properties such as moisture resistance and particle–particle interaction, may be dispersed around the lactose carrier by mechanofusion. This approach may be described as a highly energetic dry-coating process designed to mechanically fuse the guest force-controlled agent onto the carrier by solid sintering, thereby forming complete nano-structured layers around the particle [89]. The Mg st is then mechanically smeared over and fused onto the lactose particles. The mechanofusion method provides greater control of the shear and compressive forces [89]. Scanning electron microscopy (SEM) evaluation has shown that the mechanofusion process has made the surfaces of Lactohale 100 and Pharmatose 325M smoother and their shape rounder (Fig. 4). The sizes of the lactose particles showed little change through the mechanofusion process when examined by laser-diffraction analysis However, the surface properties of Lactohale 100 and Pharmatose 325M changed greatly [87]. These changes in surface morphology allow a reduction of the interactions between the drug and lactose. The drug particles may therefore be detached from the lactose surface more easily, and aerosol generation may be improved. More effective aerosolisation and a considerable time reduction in the powder-bed fluidisation and entrainment may be achieved.

A number of studies, as can be seen in Table 2, have also reported formulation strategies to improve the drug delivery into the lungs. Many of these studies have focused on improving dispersion by optimising drug–carrier interactions, and in contrast to mechanofusion, these techniques may be easily used in industrial development and production of DPI formulations.

El-Sabawi *et al.* demonstrated superior aerosol performance for a low-drug-dose formulation after decreasing the roughness of lactose carrier (63-90 μm) using controlled surface dissolution by temperature, which resulted in a decrease in the active sites responsible for the drug adhesion at a low drug level [90]. Lactose particles have also been treated with aqueous ethanol solution (70% v/v) to dissolve the protuberances on the particle surfaces and produce particles with smooth surfaces. The average adhesion force between the surface-treated lactose and drug, salbutamol sulphate, was significantly lower than that of the powder mixed with the untreated lactose carrier. Therefore, the degree of separation of the drug particles was improved [91].

The effect of covering the surface of lactose carrier particles with sucrose tristearate, a hydrophobic lubricant, has also been investigated. Compared with the powder mixed with uncovered lactose carrier, the *in vitro* inhalation properties of the powder mixture prepared with sucrose-tristearate-covered particles were significantly different, showing an increase of salbutamol sulphate FPF with the percentage of sucrose tristearate added [92]. In another study, the lactose carrier particles were coated with hydroxypropyl methyl cellulose (HPMC). The results showed that the *in vitro* inhalation properties of salbutamol sulphate increased with the surface-coating time [93].

The surface morphology of α-lactose-monohydrate particles has also been modified by a wet-smoothing process performed in a high-shear mixer using hydroalcoholic solutions [94]. Successive wetting and drying steps were performed on the lactose powders while rolling in the mixer's cylindrical bowl to modify the surface of the lactose carrier. The wet-smoothing process flattened the surface and rounded the edges of the lactose particles. In comparison with the original lactose, an improvement in powder packing and flow properties was observed. When the process was performed in the presence of a ternary agent such as Mg st, the smoothing was improved [94]. The engineering of lactose carrier surfaces using the

particle-smoothing process has also been evaluated with formulations containing beclomethasone dipropionate. The median separation energy (measured by atomic-force microscopy (AFM)) between the drug and the carrier decreased, with values of 26.7, 20.6 and 7.7 μJ for untreated lactose, particle-smoothed and particle-smoothed with Mg st samples, respectively. The FPD showed a significant increase for the lactose processed with Mg st of 102.0 ± 16 μg compared with 24.2 ± 10.7 μg for untreated lactose [95].

While the approaches described above are promising, the safety of hydrophobic excipients such as Mg st and sucrose tristearate for use in the lungs has yet to be established because their clearance mechanisms from the lungs are not well understood. Moreover, problems concerning drug-carrier separation may also be encountered (Tab. 2).

In conclusion, it is important to consider the effect of each technique on the material. Spray-drying and SCF methods offer more flexibility and possible control over morphology and size, but they occasionally yield amorphous material or undesired polymorphs. Milling remains the process of choice for micronising lactose in general and lactose for inhalation in particular because it is simpler, more predictable, easier to scale up and less expensive. However, spray-drying or mechanofusion-processing techniques, among others, are alternatives for the formulator to consider when milling or sieving does not produce the desired results. Interestingly, manufacturers are often willing to produce customised lactose for inhalation. It is then possible to obtain a desired lactose size range with a selected production method such as milling, sieving and spray-drying. Therefore, there is a need to evaluate deeply the main properties of lactose particles involved in aerosol generation and performance for each characterisation method.

3.1.4. Powder mixing

Mixing is an important step for the preparation of carrier-based formulations, such as binary and ternary mixtures between drug particles and coarse lactose carrier or between drug particles and coarse and fine lactoses, respectively) [7, 96]. An optimal mixing is required to obtain drug uniformity, especially for low-drug-dose formulations containing micronised drug particles. In the case of cohesive powders, such as those encountered in dry powder formulations for inhalation, the presence of small drug particles in combination with coarse lactose particles promotes the formation of a stable ordered mixing, in which the drug particles adhere to the larger particles that act as carriers [97]. However, in the case of ternary mixtures, where a certain proportion of fine excipient is added, some mixing issues are encountered, such as agglomeration (formation of fine and/or drug clusters due to cohesive properties of these small particles) and segregation (or demixing, characterised by the separation of the coarse particles from the fine particles induced by differences in particle size, shape and density or by agglomeration of the particles) [7, 96]. In fact, the fine excipient, which increases the aerosol performance by promoting the adhesion of drug particles to sites with lower energies than the active sites of carrier, decreases adhesion and therefore affects drug uniformity [7, 96]. For an optimal dry-powder formulation, a balance is necessary between adhesion forces that are sufficient to guarantee drug uniformity and a blend that is stable for handling but weak enough to quickly release drug particles from the carrier during inhalation. Consequently, the segregation rate occurring during the mixing could be an interesting factor in predicting the aerosol-dispersion performance [98].

The theoretical aspects related to powder mixing and mixers have been well known since the development of solid and semi-solid dosage forms [99-101]. An optimal mixing depends on the optimisation of the container filling to guarantee sufficient expansion of powder bed, the mixer and powder characteristics, and the mixing conditions. The mixers are based on one or

more of the following mechanisms: 1) convection, which is the movement of groups of adjacent particles from one place to another within the blend, 2) shear, which is the change in the configuration of ingredients through the formation of slip planes or shearing strains within a powder bed, and 3) diffusion, which is the redistribution of individual particles by their random movement relative to one another [100]. Mixers can be classified into segregating mixers and non-segregating mixers [101]. The choice of mixer depends on the tendency of the powder blend to segregate and to form agglomerates. For mixtures containing a powder blend that promotes particle separation, a non-segregating mixer must be used, while any type of mixer can be used for a mixture that does not suffer from demixing [101]. In the case of agglomeration due to the cohesiveness of the smaller components, additional stress (shear) is required to break agglomerates during mixing. Therefore, high-shear mixers are frequently used to prepare premixes of cohesive drug substances. An optimal mixing time is required to obtain a homogeneous blend. Increasing the mixing time may improve the homogeneity of a non-segregating mixture but not necessarily that of a segregating mixture. The use of pre-blending steps, i.e., where drug is blended with a small amount of excipient, could reduce the total mixing time. In contrast, the achievement of a multicomponent mixture could increase the mixing time to reach homogeneity [101].

Sebti *et al.* tested different mixers at different rotational speeds (low, medium, high) to find the optimal conditions for the mixing process of a low-drug-dose ternary mixture (1:499 w/w) of fluticasone propionate containing an 80:20 blend of coarse and fine lactoses [96]. The blend corresponded to an optimised coarse-to-fine ratio in terms of aerosol performance after having varied this ratio between 0:100 and 100:0. However, the drug uniformity was affected by the increase in fine excipients, which suggested the need to find a mixer based on a suitable working mechanism and to optimise the mixing conditions. The tested mixers were a standard planetary mixer, applying relatively high shearing forces; a usually three-

dimensional motion mixer used in inhalation research, which is characterised by moderate shearing force and efficiency in mixing powders with different densities or particle sizes; and a high shear mixer, characterised by its higher capacity to break/overcome cohesive agglomerates. The study revealed the difficulties in achieving a homogeneous mixture due to the presence of fine lactose with the three-dimensional motion mixer and the need for "powerful" shear mixers such as planetary or high-shear mixers that break down the agglomerates so that the adhesive forces (drug-coarse carrier) exceed the cohesive forces (drug-drug or drug-fine lactose). In addition, the mixing conditions must be moderated to avoid particle separation or demixing and the generation of dust [96]. Jones *et al.* tested the effect of different concentrations (0.5-4.5% w/w) of salbutamol sulphate, mixing time and blending order (drug and lactose carrier first, then lactose fines versus lactose fines and carriers first, then drug) on the aerosol performance [102]. The drug concentration and the blending method influenced the relationship between blending order and aerosol performance. They found that low-dose concentrations are dependent on the blending order, while higher dose concentrations are not affected by this factor. The authors suggest that not all formulations use the active-sites hypothesis, which applies the blending order, and that some of them depend on agglomerates and tensile-strength hypotheses (see Section 4.1.2.2). Further work is required in the area of inhalation to investigate the complex interactions between all the variables of the formulation and blending process to produce DPI systems with optimised performance.

3.2. Lactose characterisation methods

As explained before, particle properties (e.g., particle size, size distribution, shape and density), electrostatic charges, ambient RH and surface properties (i.e., roughness, surface

energy distribution and amorphous content), play important roles in the behaviour of the total powder population during the different steps of manufacturing and inhalation (i.e., metering, fluidisation and deagglomeration). The consequence of the interplay between these factors on the *in vitro* aerosol performance of lactose carrier-based dry powders is characterised by the assessment of fine particles described in section 2.1. To optimise the lactose carrier-based dry powders for inhalation, specific methods of characterisation are needed to evaluate the impact of each factor involved in aerosol performance. The primary bulk and surface powder characterisation methods used to evaluate lactose or drug particles alone and as the entire dry powder formulation will be described.

3.2.1. Bulk powder characterisation

3.2.1.1 Particle size

Laser diffraction is the most widely used technique for particle size analysis. The instruments are easy to use and particularly attractive for their capability to analyse particles over a broad size range in a variety of dispersion media. Calculations are based on the theory of equivalent spherical diameter to determine particle sizes in which the diameter of a theoretical sphere that would produce the same signal as the studied particles is considered [103]. As opposed to the impaction technique, laser diffraction provides data on geometric, rather than aerodynamic, diameter. One of the most commonly used size determination parameters is the volume median diameter, D(0.5), which is the size (μm) of particle below which 50% of the sample lies. D(0.1) and D(0.9) are the sizes (μm) of particles below which 10% and 90% of the sample lie, respectively. They are useful in evaluating the average size or PSD of the sample. The PSD of individual particles can be measured in a wet or dry sampling system. In the wet sampling system, lactose is generally dispersed in a solvent system (e.g., chloroform

or ethanol) that avoids any particle solubilisation. For dry sampling, moderately to strongly aggregated powders are dispersed by pressures between 3 and 5 bars without breaking the particles [104]. This method is widely used for the determination of lactose batch PSDs (Table 1).

Recently, new laser diffraction systems that can determine the size distribution of particles during aerosol generation have been developed (Malvern, U.K.; Sympatec, Germany). Specifically, the size properties of DPIs can be measured under simulated breathing conditions. In such a system, a laser beam crosses the end of the metal throat of an impactor [105-107]. The entire assembly is a closed system that allows for a controlled airflow rate in the measurement zone. The difference between classical and new laser diffraction systems is the particle dispersion capacity of the two methods. The compressed air values applied in the classical dry dispersion unit allow all of the agglomerates to break down, whereas the air flow generated in DPI systems is much lower and does not permit the deagglomeration of all particles [107]. Different correlations between geometric and aerodynamic size data have already been demonstrated. For a flow rate, specific inhalation device and drug formulation, the FPF can be predicted from measurements obtained from the laser diffraction technique [107].

3.2.1.2. Drug distribution uniformity

After a processing step, such as mixing the drug and coarse lactose with or without fine lactose particles (see Section 3.1.4), the uniformity of the drug content, which could be affected by the presence of fines, has to be assessed by appropriate sampling and analytical methods. Sampling methods should include using the appropriate sample size, sampling thief, sampling locations according to the blender used and sufficient sample numbers to be

representative of the blend [100]. The average drug content and variability in drug distribution in the drug-carrier blends, expressed by the relative standard deviation (RSD) between samples, are used to quantify the homogeneity of the powder. Generally, a minimum of 10 blend samples of 1-3 times the dosage unit weight must be taken at different locations according to the blender used, and the mean of the blend samples must be between 90.0 and 110.0% of the theoretical drug content with a RSD ≤ 5% to have an acceptable degree of homogeneity [108]. In the last decade, methods based on the implementation of process analytical technology that determine blend uniformity in process (i.e., without removal of samples from the mixer) have been developed and are primarily based on near infrared spectroscopy [109-112]. These eliminate many of the problems associated with blend sampling error and bias. In addition, the large increase in the sensitivity of these analytical methods should benefit the evaluation of the distribution of drug particles on the carrier surface, improving mixture homogeneity and aerosol generation control.

3.2.1.3. Crystalline state

The solid state of each component in a formulation (i.e., drug and excipient) is important in determining physical and chemical stability as well as the pharmaceutical and therapeutic performance of the drug product [113]. A crystalline system is defined by the intermolecular arrangement and spacing (i.e., bond lengths and bond angles) of the unit cell, which gives a specific diffractogram pattern in X-ray powder diffraction (XRPD) analysis [114]. Polymorphism is the ability of a chemical solid to exist in more than one crystal form. Polymorphs of the same chemical product are not physically equivalent in terms of energy states, which may result in modifications of stability, melting point, hygroscopicity, density and solubility. Methods used to define and differentiate polymorphs have been discussed by Rodriguez et *al.* [115]. Chemical compounds can be produced (e.g., by spray-drying) in an

amorphous state, also called the “disorded state”, where molecular organisation is not ordered. The amorphous state is thermodynamically unstable and tends to minimise its Gibbs free energy by transitioning to lower energy states (e.g., by crystallisation). Classical techniques, such as XRPD, are used to determine crystalline and amorphous content with typical limits of quantification (LOQs) of 5-10% [116]. Other techniques used to determine amorphous contents have been reviewed by Shaw et *al.*: thermal analysis methods (differential scanning calorimetry (DSC), modulated temperature DSC, isothermal microcalorimetry, and solution calorimetry), gravimetric analysis methods (dynamic vapour sorption (DVS)) and spectroscopic methods (infrared spectroscopy, near infrared spectroscopy, and Fourier-transform Raman spectroscopy) [116]. Methods for determining low amorphous content are discussed in section 3.2.2.4.

3.2.1.4. Moisture content

As discussed before, RH has an impact on various adhesion forces, such as capillary and electrostatic forces and surface morphology. The uptake and loss of moisture in a powder is important and can be determined by DVS, as discussed in depth by Kontny and Zografi [117]. The Karl Fisher volumetric titration method and thermogravimetric analyses are typically performed to determine total moisture content [35, 113].

3.2.1.5. Powder flow properties

Powder based on micronized drug particles possesses poor flow properties that are improved by the mixing of these micron-sized particles with large particles acting as a carrier. Powder flow plays a fundamental role in metering, fluidisation and dispersion of the dry powder and depends on intrinsic factors affecting particle adhesion and cohesion, including particle size,

shape, density, porosity and surface roughness as well as extrinsic factors, such as moisture and triboelectric charges [113]. European Pharmacopeia recommends determining powder bulk characteristics, such as the angle of repose, the compressibility index (CI), or the Hausner ratio (HR), to define powder flowability. The static angle of repose (α) is the three-dimensional angle, relative to the horizontal base, assumed by a cone-like pile of material; it is related to interparticulate friction or resistance to movement between particles. Generally, an angle of repose less than 40° indicates a free-flowing powder, and one greater than 50° indicates poor flow properties. This is not an intrinsic property and is therefore highly dependent on the method used to form the powder cone. As reviewed by Hickey et *al*., several studies have shown that the angle of repose increases with an increase in surface roughness/irregularity, a decrease in particle size, the addition of fines [102], a decrease in bulk density, or a decrease in particle sphericity [113].

The dynamic angle of repose (θ) is the angle relative to the horizontal formed by a flowing powder contained within a cylinder with a transparent, flat cover on one end and rotated at a specific speed determined by a rotating drum method. It represents the balance between internal forces and gravity and is generally higher than the static angle of repose due to variations in powder aeration. Chaos analysis of the dynamic angle of repose has been developed to characterise the powder's flow properties. The latter is correlated with the ease of particle separation (r^2 = 0.9912) and aerosol performance (r^2 = 0.9741), characterised by impact-force separation and inertial impaction, respectively [118]. The study of powder avalanches (i.e., when the balance between cohesion and gravity is broken) produced under dynamic conditions may give information regarding static flow measurements; the fluidity index, which illustrates the resistance of a powder to initiating movement during an avalanche and is related to the CI; and the cohesion index, which provides information on the capacity of the powder to be agglomerated [119].

CI, or Carr's index, has been proposed as an indirect evaluation of powder flow because it is directly affected by variations in bulk density, size and shape, surface area, moisture content and cohesiveness of materials. CI and the closely related HR are calculated either by taking the difference between tapped and bulk densities, expressed as a percentage of the tapped density, or by taking the ratio of tapped and bulk densities, respectively. Poor flow (i.e., CI > 20% or HR > 1.25) is expected to be related to compressibility; the more compressible a powder is, the more porous and the cohesive it must be. CI has commonly been measured in dry powders for inhalation to determine their flowability [90, 96, 98] and has been determined for inhalation grades lactose available on the market (see Table 1). Like the angle of repose, the CI and HR are not intrinsic powder properties and are thus dependent on the methodology used.

3.2.2. Particle surface characterisation

3.2.2.1. Surface area

The specific surface area of a powder is the surface area per unit mass (m^2/g), and consequently, it will depend on both the particle size and the surface roughness of the powder particles. Generally, the larger the surface area, the bigger the adhesive forces are. Therefore, a sensitive and accurate method for determining surface area is important and usually performed by a BET gas adsorption isotherm method in which the volume of gas, generally nitrogen, adsorbed to the powder surface at a given pressure is measured and related to a surface area [114, 120]. Calculation of the surface area based on the particle volume distribution should be avoided because this assumes that the particle shape is spherical and fails to account for the deep texture of the particle surface.

3.2.2.2. Surface morphology

Surface morphology and geometry are other important factors involved in aerosol performance, as described in section 2.2.2. They are visualised by SEM with nanometer-scale resolution. The magnification obtained with SEM ranges from 20-100.000×. The surface morphology can also be studied at different relative humidities using an environmental SEM [44]. AFM, described in section 3.2.3.1, is also used to characterise the three-dimensional topography of the particle surface using the tapping mode (i.e., non-contact mode) [113]. Variation in particle morphology within a given area (usually 10×10 μm^2) is determined by calculating the root mean square roughness [113]. Environmentally controlled AFM has been used to observe and monitor the crystallisation of lactose from an amorphous state at different relative humidities [121]. The particle roughness has been also evaluated by taking the ratio of the external specific surface area determined by BET adsorption to the theoretical surface area calculated from the particle volume distribution, assuming a spherical shape of particles [34, 122]. Other studies have used the surface fractal dimension to define surface corrugation, which is determined by light scattering techniques [35]. Adi et *al.* suggest using scanning white-light interferometry to determine the roughness of a sample [123]. The sub-nanometer resolution over large areas (e.g., 50×50 μm^2) makes it possible to image multiple particles with step heights as small as 0.1 nm.

3.2.2.3. Electrostatic charges

As seen in section 2.2.1.3, the triboelectric charging of dry powders for inhalation influences the magnitude of adhesion forces and plays a role in particle deposition in the lung. In general, the net charge of a powder is measured with a Faraday cage because it is simple and reliable [22]. Such a system has been used to evaluate the influence of the contact surface and RH on the triboelectrification of dry powders for inhalation [24]. An open Faraday cage

connected to an airflow system has been used to study the effect of particle morphology on triboelectrification [27]. A charge measurement system with an impaction stage upstream of the Faraday cage has been developed to evaluate the influence of triboelectrification on aerosol performance by considering only particles with an aerodynamic size in the respirable size fraction [32]. Chow et *al*. developed a Faraday cage connected to an airflow system to study the electrostatic charges generated inside of an inhalation device during aerolisation [29]. An alternative method has utilised an electrostatic grid-probe to determine aerosol electrostatic charges [124].

However, Faraday cage methods have some limitations because the positive and negative charges of the different powder size fractions are measured as a net charge. To differentiate between the polarity and magnitude of charges carried by different particle size fractions, other techniques have been used, including the electrical low-pressure impactor (ELPI^{TM}). The ELPI^{TM} was originally developed as a real-time size analyser for 30-nm to 10-µm particles sizes [125]. It is composed of a unipolar corona charger, where the particles are charged before being separated through the 13 stages of the cascade impactor at 30 l/min according to their d_{ae} and simultaneously detected by a multichannel electrometer that measures the charges carried by the collected particles from each electrically insulated stage [126]. Glover and Chan adapted the ELPI^{TM} to assess electrostatic charges from an aerosol device with the corona charger switched off [127]. The ELPI^{TM} has subsequently been tested with drug-lactose carrier-based formulations delivered by DPIs to determine the influence of RH, lactose type (i.e., milled or sieved), capsule material, and inhaler on the triboelectrification of dry powders [30, 33]. However, the ELPI^{TM} is limited to assessing the electrostatic charges of particle size fractions at a flow rate of 30 l/min. To overcome this limitation, a more recent system has been developed, the electrical next generation impactor,

which can assess electrostatic charges carried by the different particle size fractions at different flow rates [128, 129].

The electrical-single particle aerodynamic relaxation time analyser based on the laser Doppler velocimeter technique could also be used to assess electrostatic distribution and particle size on a single-particle basis [130, 131], and the direct observation of single particle electrostatic charging by AFM has also been reported [132].

3.2.2.4. Surface amorphous content

Small degrees of disorder, such as amorphous regions, may arise on the surface of the lactose carrier during processing (e.g., milling) and significantly affect the performance of the powders, even at very low levels. Within amorphous regions, substantial absorption of water occurs, and it can cause physical and chemical instabilities [133]. Therefore, an accurate and reliable measurement of low levels of amorphous content is essential. XRPD is a sensitive technique for determining amorphous content with a typical LOQ of 5-10%. However, Chen et *al*. have reported an improved XRPD technique with a LOQ for amorphous lactose decreased to 1% [134]. Other techniques have been used to study the amorphous lactose content of powders: Raman [135-138] and near infrared spectroscopy [139], high speed DSC [140], isothermal microcalorimetry [141, 142] and solution calorimetry [135, 143, 144]. However, their sensitivity is not high enough to be able to measure the very low levels of amorphous lactose present in the crystalline grades that are used for inhalation (i.e., <1%).

A powerful way to investigate surface properties is through vapour sorption studies, which preferentially probe amorphous regions that are characterised by an increased molecular mobility that enhances the vapour absorption behaviour [145]. DVS is a gravimetric approach based on the water mass difference before and after crystallisation of the amorphous content to α-lactose monohydrate and is generally used to quantify amorphous content because the

technique is very reproducible, with an LOQ of approximately 0.5% [146]. However, the crystallisation of amorphous lactose to other crystalline forms, such as β-lactose or anhydrous α-lactose, may occur and distort the determined amorphous content. In contrast to the crystallisation approach, a new gravimetric vapour sorption technique that is based on the moisture sorption isotherms of amorphous lactose has recently been reported [147]. The moisture sorption isotherm of lactose with a known amorphous content was determined and fitted to a BET formula. A linear relationship was found between a fit parameter (i.e., the monolayer water content) and the amorphous content, allowing the amorphous lactose content to be quantified accurately over the entire range of 0-100% amorphous lactose, including the low-level range of 0.1%-1% [147]. Other authors have used a gravimetric vapour sorption method based on organic vapour sorption to determine unknown amorphous contents with an accuracy of about 0.05% [148]. Murtomaa et *al.* (2002) suggest using the triboelectrification data, which is a surface sensitive phenomenon, to quantify low surface amorphous contents (<1%) [26].

3.2.2.5. Surface energetic distribution

The surface energetic distribution plays an important role in adhesion and the separation of drugs from the carrier during inhalation, as discussed in section 2.2.2. The total surface free energy can be represented as the sum of the dispersive surface free energy due to van der Waals forces and the specific (i.e., nondispersive) surface energy related to acid/base interactions that contributes to interparticulate forces independently [17, 149].

The surface energy of particulate samples is difficult to measure, and traditional liquid contact angle methods are not well-suited to powdered samples. It has been argued that vapour sorption methods, such as inverse gas chromatography (IGC), are preferred for studying the powder surface energy [150]. The principle of IGC is to pack a sample of powder into a gas-

chromatography column and determine the net retention time within a series of well-characterised non-polar (usually alkanes) and polar gaseous probes injected at infinite dilution. This results in interactions between the probes and the high-energy sites of the powder surface. The net probe retention time resulting from the interaction strength between the gaseous probes and the powder surface can be translated into the retention volume, which allows determination of the dispersive-surface free energy (γ_S^D, expressed in mJ/m^2) and the surface acid/base properties (K^A and K^B, expressed in kJ/mol) [151-153]. This technique is indirect, non-destructive, sensitive, reproducible and capable of evaluating not only individual particles or particle contact regions but also the entire powder surface. Cline and Dalby standardised the units of these components in mJ/g (i.e., γ_S^D , K^A and K^B) and correlated them to *in vitro* aerosol performance [154]. Several reports have used IGC to detect surface energetic variations in lactose batches that are not detected by other techniques [155]. IGC has been used to evaluate the influence of RH on the surface energy of different processed lactoses [38, 43, 98, 156], examine the influence of lactose pseudopolymorphism on surface energy and its correlation with *in vitro* aerosol performance [157] and understand the impact of mechanofusion with an additive on the lactose carrier surface and *in vitro* aerosol performance. The latter seems to be primarily influenced by variation in the acidic parameter [87, 88]. Moreover, IGC has been compared with the traditional liquid contact angle technique, the Wilhelmy plate method [158]. The IGC technique has been used to relate results concerning solubility parameters; it allows quantification of the solid-solid interactions involved in particle adhesion and cohesion and therefore correlates the difference between the strengths of adhesive and cohesive interactions to the *in vitro* aerosol performance [159]. IGC measurement conditions have been evaluated to study the surface energy of lactose monohydrate by Planinsek et *al*. [160]. The adsorption energy values calculated by molecular modelling and measured by IGC were in quantitative agreement with each other. Molecular

modelling helps to relate the dispersive and specific surface energy to the functional groups present on the surface [161].

Recently, a new IGC method at finite concentrations has been used to study the entire surface energy distribution (i.e., high and low energy sites) and distinguish the heterogeneity of this distribution in different processed lactose carriers [38]. Ho et *al*. have used this method to observe the influence of fine lactose and small changes in crystal habits on surface energy distributions [162].

3.2.3 Interparticulate adhesion forces

Interparticulate interaction forces (e.g., van der Waals, capillary and electrostatic charges) are dependent on different factors, such as crystalline state, particle size, PSD, shape, density and surface properties, including roughness, amorphous content and surface energy distribution. However, RH is also involved in the adhesive forces between drug particles and the lactose carrier. The magnitude of these forces may be directly measured at the individual particulate level by AFM, which allows researchers to estimate and understand the impact of some of these factors on adhesion. It can be determined for the entire bulk powder by indirect methods to evaluate the magnitude of adhesion force resulting from the interaction of all the above-mentioned factors.

3.2.3.1. Direct interparticulate adhesion force determination

Determination of the interparticulate interactions between a drug probe and its carrier substrate is made at the single-particle level by AFM using the colloid probe microscopy technique [11]. Briefly, the micronised drug (i.e., the probing tip) is attached to an elastic cantilever that is vertically deflected proportional to the adhesion forces experienced by the

tip due to the approach and retraction of the carrier substrate mounted on a piezoelectric tube. The vertical displacement (Δz) of the cantilever is converted into adhesion force (F_{ad}) by applying Hooke's law ($F_{ad} = k\,\Delta z$), where (k) is the cantilever spring constant. The mean geometric adhesion force and geometric standard deviation values are determined from a lognormal force distribution. AFM has sub-angstrom scale resolution sensitivity and can detect forces on the order of picoNewtons (10^{-12}N).

Initially, researchers studied the adhesion force exerted by different lactose carriers on a conventional spherical probe tip [163] and progressively used AFM to evaluate the adhesion force between micronised drugs and lactose carriers or other surfaces [164-166]. Subsequently, the influence of RH on the topology and adhesion force between drug probes and lactose carriers was studied [21, 41, 167]. The influence of fine particles on the adhesion forces between the drug probe and lactose has also been determined [168, 169]. Adi et *al*. evaluated the influence of the morphology of different corrugated-level drugs on adhesion force and *in vitro* aerosol performance [170]. Young et *al*. used AFM to evaluate the roughness of developed lactose composite particles and the adhesion force between the drug and these carriers to find a correlation between these factors and *in vitro* aerosol performance [86]. Islam et *al*. underlined the importance of the probing tip particle size and geometry in the contact area with the roughness of the lactose used as a substrate and their impact on the magnitude of adhesion force measurements [171]. Surface forces have also been examined using the colloid probe technique with cohesive-adhesive balance [37, 172]. Overall, AFM is a very useful tool for studying the underlying mechanisms of the interparticulate interaction between drug and carrier and understanding the physico-chemical and environmental factors that govern their magnitude and may have an impact on *in vitro* aerosol performance. However, the results obtained by AFM relate only to the interaction between two particles and are limited to a small area of the sample. Therefore, this technique is not transposable to the

routine analysis of the entire blend because of cost and time considerations. Moreover, characteristics such as particle size, shape, or heterogeneous surface roughness are not considered.

3.2.3.2. Indirect bulk adhesion force determination

Indirect determination methods, such as centrifuging, sieving and impact separation techniques based on indirect drug detachment quantification, have been used to evaluate bulk adhesion forces in powders [173, 174]. The advantage of these methods is the possibility of measuring the interactions of a relatively large number of particles with the carrier surface; thus, these methods may result in a more representative statistical value for the entire powder. Among these indirect methods, centrifuging has been used on dry powders for inhalation [91, 175]. This method uses centrifugal force to separate adhering particles from the substrate surface. The particles are detached from the surface when the magnitude of the centrifugal force exceeds the magnitude of the adhesion force. The force necessary to detach 50% of the particles from the substrate is often used to represent the force of adhesion between the particle and surface.

Mechanical sieving has also been reported, where drug detachment was facilitated by vibration (i.e., shakes and shocks). The aim was to observe if the blend was sufficiently stable to resist to the vibrations [8, 176]. An air jet sieve method has also been used that allows assessment of the separation characteristics of a micronised drug from carrier particles in an air stream by measuring the percentage of drug retained on the carrier [8, 91, 176]. Results from this method have a linear relationship with *in vitro* aerosol performance [8, 176]. The latter methods are simple and easy to perform, making it possible to characterise adhesion,

forecast blend stability and drug detachment from lactose, and predict the aerodynamic behaviour of the drug.

Lactose carrier-based dry powders for inhalation are highly dependent on the physico-chemical characteristics of the lactose particles in adequately delivering drug particles to the lungs. Despite the fact that the lactose (both anhydrous and monohydrate) used in the inhalation field fulfils the specific lactose requirements described in pharmacopoeias, intra- and inter-batch and supplier variations are observed [69]. It would stand to reason that further or more appropriate characterisations should be defined to guarantee the functionality of lactose when it is used for inhalation [69]. As has been extensively described in this work, surface amorphous content, energy surface distribution and surface area are all factors that could be varied during the lactose manufacturing process and are significantly involved in the adhesion and dispersion properties of the lactose. These factors should be evaluated by the appropriate characterisation methods that take into account the entire sample. Currently, vapour sorption methods (e.g., BET gas adsorption isotherm, DVS and IGC) seem to be the most appropriate tools to meet these requirements.

4. Influence of lactose characteristics and distribution on aerosol performances

4.1. Use of lactose as a "carrier"

Because micronised drug particles are cohesive and have poor flow properties, lactose particles are most often incorporated as a carrier with the micronised drug powder to make the

powder blend less cohesive and free flowing. In the particular case of DPIs, the carrier consists of coarse, inert particles onto which the drug particles adhere to improve powder fluidisation, as illustrated in Figure 6A [14]. During aerosol generation, drug particles leave the surface of the carrier and penetrate into the lung. It has been suggested in several earlier studies that the efficiency of a powder formulation is highly dependent on the selection of the carrier, its quality, source, size distribution, morphology and surface properties as well as the presence of a ternary agent [6, 177, 178]. In fact, a direct link between one or more physico-chemical parameters and efficiency could not be established [122].

Because many DPI formulations are interactive blends of micronised drug and larger carrier particles, drug-to-carrier adhesion properties are important design considerations. If particles adhere strongly, the inspiratory airflow of the patient during DPI actuation may be insufficient to separate micronised drug from the carrier particles, which may result in poor and/or variable delivery to the lung. Understanding the adhesion forces between carrier and drug would aid in drug particle manufacturing and help determine the type of excipients to be used in interaction optimisation [113].

Therefore, the materials that can be used as the carrier should be readily available at an acceptable pharmaceutical grade, chemically and physically stable, and inert to the drug substance. Because the carrier particles are the main component in such formulations, any change in their physico-chemical properties may affect the drug's lung deposition. Most importantly, the material should clear readily from the airways and not exhibit harmful effects on the respiratory tract.

More than three-quarters of the most common DPIs on the market, such as Beclophar®, Flixotide®, Relenza®, Seretide®, Spiriva® and Symbicort®, use lactose as a carrier. The reasons for this are as much historical as they are physico-chemical/pharmaceutical in nature.

Lactose was used for a long time as an excipient in oral dosage forms before being deployed in DPIs. It has an established safety and stability profile as well as different manufacturing processes with tight controls over purity and physical properties. It is also easily available in different grades and inexpensive [179]. Published inhalation studies in humans have shown no local effects of lactose exposure by inhalation [180]. Because the particles of lactose in these preparations are generally larger than the respirable range, the bulk of lactose will be deposited mostly in the mouth and throat where it is either hydrolysed by bacterial enzymes present in normal saliva or swallowed and subsequently metabolised by intestinal enzymes. Any lactose deposited in the lungs is rapidly absorbed, metabolised by the intestinal epithelium and principally excreted in urine [181]. Furthermore, in contrast to oral administration, lactose swallowed at the levels present in inhaled preparations (i.e., up to 25 mg) is unlikely to present problems, even in patients with lactose intolerance [180].

4.1.1. Lactose in binary mixtures

Although the choice of the excipient may seem restricted and simple in the development of DPIs, the selection of lactose grade and PSD is crucial and significantly influences the efficiency of the final product [6, 9, 10]. In light of the limited number of approved DPI excipients, suppliers are increasing their range of lactose products for use in DPI applications (e.g., DMV-Fonterra, Domo, Meggle, Sheffield-Bioscience). For example, sieved or milled lactose in a wide spectrum of PSDs with various particle shapes and surface properties are now commercially available (Table 1).

There are differences between milled and sieved batches of α-monohydrate lactose. Generally, the non-milled raw material has resulted in lower aerosol generation compared to the milled materials. A major reason for the different aerosolisation behaviour can be seen in

the modified surface structure of the lactose crystals. The un-milled quality contains few, fine particles and exhibits partially rough-structured surfaces, whereas the milled material is made up of more fine lactose particles produced during milling. As the corroded fine lactose is preferentially placed within the edges and clefts of the larger lactose crystals, the drug can adhere to the smoother surface of the crystal where it can be removed easily [122]. These fines contribute to aggregation of the powder under static conditions but behave as primary particles during motion, allowing the milled powders to exhibit more uniform flow than the sieved batches. For example, the performance of milled lactose monohydrate exceeded that of the sieved batches in delivering albuterol sulphate [31].

The selection of the polymorphic form of lactose is important because they each have different particle morphology, crystallinity and physico-chemical properties (i.e., solubility, melting point, density and hardness) [6]. It has been shown that the crystallinity of lactose plays an important role in aerosol generation. Having high-energy surfaces, amorphous lactose exhibits strong adhesive interactions with drug particles, leading to low inhalation efficiency [34]. For example, studies have suggested that β-lactose results in significantly poorer aerosolisation performance than α-lactose [157]. Anhydrous lactose has a distinctively different shape from that of α-lactose monohydrate, the former being comparatively rounder in shape. The α-lactose crystals have the shape of a tomahawk, which is the shape of a lactose crystal allowed to grow to maturity [6]. Anhydrous lactose consists of rougher particles, which reflect the roller-dryer method of manufacturing and result in the formation of microcrystallite assemblies rather than distinct crystals, as visualised in Figure 5 [182]. To investigate the possibility of using inhalation-grade, anhydrous lactose in carrier-based formulations, studies with salbutamol sulphate and budesonide have been conducted [6, 182]. Larhrib *et al.* observed more efficient drug delivery from anhydrous lactose, which may be partly attributed to the relatively higher concentration of fine lactose in this grade of carrier

(see Section 4.1.2.1), even though it exhibited a rougher surface [6]. Anhydrous lactose is known to be more brittle than α-lactose monohydrate due to the removal of the water of crystallisation, leading to the partial disruption of the crystalline order [183]. During air-jet sieving or milling, particle friction and collision may be more likely to induce fragmentation of anhydrous lactose than in α-lactose crystals. However, Pitchayajittipong *et al.* have demonstrated that anhydrous and monohydrate grades of lactose from the same supplier appear to exhibit similar fluidisation energies and FPD [182]. Nevertheless, anhydrous lactose absorbs more water at high RH than lactose monohydrate, which is a consequence of the conversion of anhydrous lactose to the monohydrate form [182]. Therefore, the grade and/or source of lactose may have a substantial effect on drug delivery, and care should be taken to establish appropriate quality control parameters when selecting a carrier grade.

Consequently, α-lactose monohydrate is the crystalline form most commonly employed as a drug carrier in DPIs. It is possible to obtain it in a wide spectrum of PSDs (Table 1). The effect of carrier particle size on drug deposition is well documented. A reduction in carrier particle size has been shown to improve the aerosolisation of various drugs [66, 184]. For example, a study conducted on salbutamol sulphate and lactose carriers with major differences in the PSD showed an increase in FPF with a decrease in carrier particle size. Size fractions smaller than 90 µm produced a higher FPF than size fractions above 150 µm [177]. The inverse relationship between the carrier, D(0.5), and FPF was confirmed by a correlation (r^2: 0.919), showing that a decrease in the carrier particle size improves aerosol drug generation [177]. It has been demonstrated that powders with a median diameter of more than 100 µm may not be entrained effectively by the air stream generated into the device [185]. Moreover, with increasing mean carrier diameter, the flow properties improve, whereas the HR decreases. This causes the inertial and frictional forces within the powder to increase during mixing. It has also been shown that carrier surface rugosity and impurity increase with

increasing mean carrier diameter and the number of active sites thereby increase in terms of multiple contact points, contact area and capillary forces, decreasing aerosol generation [186]. The use of a too small carrier will result in poor flow properties in the powder, which is one of the primary reasons for incorporating a coarse carrier within the formulation. Therefore, a typical particle size of lactose used as a carrier for inhalation aerosols is the sieved fraction, ranging between 63–90 mm [6, 40, 187]. However, one strategy for improving drug aerosolisation without substantially reducing the mean carrier size is to add a small amount of fine carrier to the binary interactive mixture composed of the coarse carrier and drug (see Section 4.1.2.1).

Besides crystalline form and particle size, surface roughness and the shape of the carrier particles have also been described as having a major influence on the adhesion of drug particles to the surface of the carriers [8-10]. Increasing the surface smoothness of lactose carrier particles has been shown to improve the potentially respirable fraction of albuterol sulphate and terbutaline sulphate [8, 188]. The correlation coefficient, r^2, of the straight line, "terbutaline sulphate FPF - Roughness", is 0.9066, displaying a good correlation between these two parameters [8]. Using lactose with a smoother surface will therefore enhance particle dispersibility, which will require a lower inspiratory effort from the patient to detach the drug from the lactose and obtain a high FPF. In fact, an increase in roughness multiplies the contact points between drug and carrier, which favours binding and, consequently, drug adhesion to the carrier. This phenomenon stabilises the blend and avoids segregation between drug and carrier, but separation of the drug from lactose becomes more difficult during aerosolisation. Interestingly, apart from surface smoothness, it was demonstrated that the shape of the carrier particles also played an important role in determining the FPF of the drug. The dispersibility of salbutamol sulphate increases almost linearly with the values of either the elongation ratio or the surface factor, with a linear coefficient, r^2, of approximately 0.90.

Thus, the particle shape of the carrier particles appears to be as important as the carrier surface smoothness in determining the dispersion and deaggreagation of salbutamol sulphate [9]. In fact, a clear relationship exists between contact area, drug carrier adhesion and aerosol performance.

Careful selection of PSD, shape and surface roughness is required to optimise aerosol performance and reduce variability in respiratory delivery. Unfortunately, there have been no strict criteria for controlling the morphological features of the carrier particles for inhalation aerosols, and variation in such factors may be one of the main causes of batch-to-batch variation in drug delivery encountered for most dry powder aerosol formulations [189]. The engineering of carrier particles to produce a precisely designed shape may provide an important strategy for improving drug aerosol generation to the lower airways by DPIs.

4.1.2. Lactose in ternary mixtures

As previously seen, the presence of fine particles produced by milling and attached to the surface of the coarse carrier influences the FPF of the formulation tested. Therefore, to allow a more accurate quantification of the effects of adding fines to a formulation, a number of studies have incorporated pre-treatment of a coarse lactose carrier to remove any pre-existing (intrinsic) fine particles by either air jet sieving or air washing lactose or by wet decantation. For example, the FPF of salbutamol sulphate decreased from 11.8%, when the original lactose was used, to 6.7% for treated lactose where more of the fine lactose was removed by compressed air treatment; moreover, the addition of 1.5% w/w fine lactose to the formulation increased the FPF to 14% [190]. Nevertheless, the possibility that the removal of intrinsic fines might induce other changes in the carrier material should be borne in mind. For example, air jet sieving or air washing might lead to the appearance of electrostatic charges on

the surface of the lactose carrier. Although lactose is practically insoluble in ethanol, wet decantation might induce surface changes in the coarse particles (for example the recrystallisation of amorphous regions or microscale morphological changes). However, in a study by Islam *et al.*, when the fine lactose, removed by wet decantation, was restored to its original concentration by the addition of micronised lactose, the FPFs were not significantly different from those of the original lactose samples [168].

4.1.2.1. Influence of fines on aerosol generation

The addition of fines to blends of coarse lactose carrier (typically the 63-90-μm size fraction) and drugs has become a subject of active research in the development of DPI powder. The fine lactose used in these investigations has typically had a D(0.5) of 4-7 μm, and the proportion added has been in the range of 1.5-20% (w/w) relative to the powder formulation [178]. Anti-asthmatic drugs, such as salbutamol sulphate, salmeterol xinafoate and beclomethasone dipropionate, have been the most frequently investigated; other drugs, including proteins, have also been used [7, 170, 177, 191].

The influence of fine lactose size on the performance of ternary formulations was investigated on various drugs, namely, salbutamol sulphate and salmeterol xinafoate. Fines of different particle size ranges (i.e., <5 μm, 5-10 μm, 10-20 μm and 20-45 μm) were employed, and those with a mass median diameter of approximately 5 μm were found to give the greatest FPF [39, 190, 192]. In fact, fine lactose may not be considered as a "carrier", but when particle size was large enough to ensure that the added lactose particles were non-cohesive and free-flowing, the added fine lactose acted as a "secondary carrier". Subsequently, salmeterol xinafoate became strongly adhered to the surface of these particles, and dispersion was decreased [83, 193]. Therefore, because the particle size of fine lactose will also

influence drug dispersion, careful control of particle size specifications for the added fines is important in DPI development.

Studies have also indicated that the aerosolisation performance of drugs improves when the amount of fine lactose particles is increased [6, 177, 194]. For example, the FPF of formoterol fumarate was found to increase with the amount of fine lactose added. This influence appears to be linear for up to 5% of fine lactose, with an increase of approximately 3% for each percent of fine lactose added. One may assume that above 5% of fine lactose, the mechanism of influence on performance undergoes a change. Competing effects, such as the saturation of activated areas on the carrier surface or the formation of drug-fines agglomerates for easier lift-off, could offer an explanation for this behaviour (see Section 4.1.2.1) [195].

However, the existence of larger carrier particles is essential in optimising the dispersion process, with maximum drug deposition achieved with carrier systems containing around 10% fine particle and 90% coarse particle concentrations. The influence of the coarse carrier on particle dispersion was only significant at fine particle concentrations of up to 10% in data collected, where the inherent, adhered fine lactose particles were likely to become the dominant driver of dispersion. There was no significant difference in FPF due to carrier size when there was an excess of fine particles (i.e., >35%) [177]. In fact, Islam *et al.* indicated that the lactose surface characteristics of different samples of lactose carriers were less important in controlling the aerosol generation of particles than the critical ratios of drug-to-fine lactose [196]. Fine lactose increased the FPF and dispersability of albuterol sulphate to such a level that all lactose batches produced a similar fraction of aerosolised drug, regardless of particle size or whether they were solvent treated. It is important to note that fines are inherently poor flowing due to an increase in their surface area and cohesive forces. The presence of more than 35% of fines tend to reduce powder flowability because fines can fit into the voids between larger particles, encouraging packing and consequent powder

densification [194]. Therefore, the amount of fine lactose needs to be kept at a sufficient level to enhance aerosol generation without decreasing flow properties. Moreover, stability testing of blends after 28 days of storage at ambient conditions has revealed a notable decrease in aerosolisation performance for the blends with high levels of fine lactose carrier. This effect can be explained by an increase in the surface area of lactose with higher fines content. Over a four-week period the powder adsorbed moisture from the ambient air led to an increase in capillary forces between drug and carrier. However, storing the blends at elevated temperature and humidity (i.e., 40°C/75% RH) resulted in a greater decrease in aerosolisation performance that could not be correlated to any particular lactose size fractions [194]. It is important to note that the use of micronised, fine particles may introduce extra amorphous content to the powder because a significant portion of micronised lactose exists in the amorphous form, which is thermodynamically unstable and will transform to the more stable crystalline form upon exposure to moisture. Such a transformation is likely to alter the performance characteristics of the bulk powder, including flowability and drug dispersion. Data obtained from the manufacturer suggest that the amorphous content for fine lactose is approximately 8% [194].

Therefore, the concentration and particle size of fine lactose have to be carefully controlled to achieve satisfactory and reproducible pharmaceutical performance from a specific device. For example, increasing the concentration and/or reducing the particle size of fine lactose is expected to improve the dispersion of drugs, but this would have a detrimental effect on the powder's flowability, mixing homogeneity and long-term stability. Optimal particle size and concentrations of fine lactose would appear to be a function of the device, drug, coarse lactose characteristics and specific requirements of a particular formulation. Given the wide variety of available devices and therapeutic purposes of inhaled drugs, it is likely to be impossible to identify a single acceptable concentration and/or particle size for fine lactose. Nevertheless,

while it is clear that the presence of fine particles is a key factor in controlling dispersion, the exact explanation for their mechanism of action is not yet known.

4.1.2.2. Hypothesis about the mechanism of fines

Two mechanisms, represented in Figure 6, have been proposed. The “active sites” hypothesis suggests that there are areas on the carrier surface that are more adhesive than others. It has been proposed that fine excipient particles preferentially bind to areas on the surface of the coarse carrier with the strongest binding characteristics, thus forcing drug particles to bind to areas with weaker binding characteristics [10]. It has been described that the fine particles are likely to fill up carrier surface irregularities (Fig. 6.B). They may accumulate in a cavity or crevice of a carrier particle, allowing the passivation of the strongest binding site, and they are then no longer available for aerosolisation. In addition, when a high proportion of fine particles is added, the formation of multiple, complete layers of fines covering the surface of the coarse lactose particles may occur, thereby hindering direct contact between drug and carrier and promoting drug particle detachment from the carrier surface during aerosolisation. This phenomenon may also decrease the cohesion of the powder by improving the slip between particles. During inspiration, drug particles are more easily liberated from the surface of the carrier particles, increasing the generation of aerosol and the proportion of drug available for inhalation [10, 39, 197].

The “agglomerates” hypothesis proposes that fine drug and excipient particles adhere to each other in the formulation, forming structures that are better aerosolised and dispersed than single drug particles [169, 191, 198, 199]. During the blending process, drug particles are distributed between the surface of the carrier and “multiplets” formed by aggregation of fines and drugs (Fig. 6.C). Upon aerosolisation, it is hypothesised that drug particles are more

easily liberated from fine particle multiplets than from the surface of coarse carrier particles. This would be expected because smaller particles have a lower degree of surface roughness, which restricts opportunities for contact at clefts and aspirates the substrate surface, resulting in lower interaction forces and more effective removal forces [51]. Because these agglomerates are subjected to weak cohesive forces, they are loose, and aerosol generation of the drug may be enhanced. Moreover, because the separation distance plays a significant role in van der Waals attraction, the presence of these aggregates in the powder increases the distance between particles, which will improve particle dispersion. Fine-particle multiplets might also be small enough (i.e., < 5 μm) to form part of the FPF without detachment of drug particles, and they are believed to travel to the lower regions of the lung [63].

The influence of the order in which carrier, drug and fines are blended on formulation performance provides evidence to support the "active site" hypothesis. Various studies have demonstrated that formulations produced by blending the coarse carrier and fines before the addition of the drug give greater fine particle delivery than formulations produced by blending the coarse carrier and drug first [7, 39]. It has been suggested that when coarse carrier and fines are blended first, the fines have the first opportunity to adhere to active sites; thus, drug particles adhere to less adhesive sites when they are added to the formulation, resulting in greater fine particle delivery. The opposite situation has been suggested to apply when coarse carrier and drug are mixed first. It has been observed that the maximum drug FPF values were obtained when a formulation of salbutamol sulphate was made by pre-blending fine lactose with coarse lactose before mixing with the drug [39]. In contrast, it was the formulation prepared by pre-blending the drug with fine lactose prior to its addition to the coarse lactose that resulted in the highest FPF after aerosolisation for formulations containing beclometasone dipropionate. In this case, it is possible that the fine lactose in the pre-blend increased the FPF

by disrupting aggregates of the highly cohesive drug, thereby promoting aerosol generation of individual drug particles [7].

However, other investigations have demonstrated that fine particle delivery from ternary formulations produced by both blending orders was equal [169, 191]. The data indicated that the effect of fine lactose on the deposition of bovine serum albumin was independent of the order of addition to the ternary powder formulation. If fine lactose particles were acting only by occupation of binding sites on the surface of the carrier particles, then addition to a binary-ordered mixture would, theoretically, have had no effect on the FPF.

Variation in interparticulate adhesion caused by the use of different drugs and carriers has been shown to influence the FPF of formulations; thus, the selection of the drug, its concentration and the blending method in a DPI formulation may influence the aerosol generation of the powder and explain the discrepancy between the different studies [102]. In fact, both theories may exist in a dynamic equilibrium. However, the mechanism of action of ternary components within DPI formulations has not been fully elucidated. Further insights into the mechanisms by which fines improve DPI performance are most likely to come from a greater understanding of the interparticulate interactions between the components present in ternary formulations and how these interactions influence the deaggregation process.

4.2. Use of lactose as an "excipient"

It is interesting to note that lactose is often used as a bulking agent and stabilising adjuvant in spray-dried formulations of sensitive drugs, peptides and proteins to provide some protection during the manufacturing process against shear forces and increased temperatures in particular [200-205]. The structure and activity of dried proteins are protected by hydrogen bonding

with lactose, which enables the production of stable particles of a size suitable for inhalation and allows a rapid release of the drug in the lung.

Lactose plays an important role in spray-dried formulations containing dipalmitoylphosphatidylcholine (DPPC) and albumin by producing small and large porous particles [206, 207]. These particles allow the incorporation of interface-sensitive peptides and have also yielded high pulmonary bioavailability of drugs [208, 209]. A typical ratio of the excipients is DPPC:albumin:lactose at 3:1:1 [210, 211]. In fact, lactose might offer the skeletal structure necessary for the formation of solid particles with a weak agglomeration tendency through spray drying [206].

Lactose has also been used as a binder of poly(lactic-co-glycolic) acid with liposomal or chitosan nanoparticles, forming nanocomposite microparticles. After reaching the alveoli, the microparticles were decomposed to nanoparticles, as lactose is soluble in the alveolar lining fluid [205, 212-214].

Due to its structure, lactose has also been used as a cryoprotectant in liposomal dry powder formulations. Hydration of liposome polar head groups with the hydroxyl groups of lactose leads to stabilisation, and, during dehydration, lactose protects the liposomal permeability barrier, reducing leakage of the drug from the liposomes [215].

Because lactose particles are often dissolved and incorporated into a blending of drugs and other excipients, the lactose physico-chemical characteristics, such as PSD, shape and surface properties, are less important than for ordered mixtures with lactose as a carrier for these applications.

4.3. Alternatives to lactose in dry powder formulations for inhalation

Other formulation strategies than the use of lactose for dry powder also exist [5]. Nevertheless, because it is not the purpose of this paper, these interesting developments will be treated briefly.

Other sugars and polyols have been tested as potential carriers and fine excipients in DPIs, such as mannitol, glucose, trehalose, dextrose, maltose, sorbitol, maltitol and xylitol [4, 66, 170, 191, 216-219]. Anhydrous glucose is already used as a carrier in the marketed product, Bronchodual® (Boehringer). The physico-chemical nature of the sugars and their hygroscopic properties have been shown to influence aerosol generation of the drug from the mixtures. The mean adhesive forces were significantly different for each of the sugars with the following decreasing order: sorbitol > lactose > mannitol > glucose [170].

In fact, excipients that modify the hygroscopic properties of drug particles may need to be considered. Recently, amino acids (e.g., glycine, alanine, leucine, isoleucine) have been shown to decrease hygroscopicity and improve surface activity and charge density of particles [220, 221]. New formulations have been developed using solid lipid microparticles that are composed of distearoylphosphatidylcholine and cholesterol, two well-tolerated components physiologically, as a pharmaceutically acceptable filler and/or carrier to improve the deposition of budesonide [222]. The same composition of lipids has also been used for coating drug particles to modify the surface properties of the particles and enhance aerosol generation [223].

It is interesting to note that the number of excipients that have been declared safe for use for pulmonary delivery is restricted. Using a non-approved excipient involves extra work, cost and time and carries the risk of rejection by regulatory authorities. Moreover, the addition of a carrier in elevated ranges decreases the dose of active drug administered. Thus, there is an

increasing interest in formulations that do not require a carrier to aid the flowability of the micronised drug (viz., “carrier-free” formulations). For example, carrier-free tobramycin powders, with the particle surface properties modified by creating a film of the amorphous drug, have been produced by spray-drying and showed high FPF [224].

5. Conclusion

Currently, the aerosol generation of drugs from DPIs are being improved by changing formulation strategy, drug and carrier particle engineering and designing new devices. Indeed, the design of a device needs to be coordinated with drug formulations so that the drugs are aerosolised during inhalation and deliver a dose to the lungs that achieves maximum therapeutic benefits.

Concerning formulation development, micronised drug particles are cohesive, and their flow properties are poor. The method of adding large lactose carrier particles into powders to improve their fluidisation has been a popular choice for DPIs because such a method is easy to apply and cost effective. The inclusion of fines in carrier-based dry powder inhalation systems is also an extensively researched area and a useful technique for the improvement of formulation performance.

The main goal in the inhalation field is to obtain reproducible, high pulmonary deposition. This could be achieved by successful carrier selection and careful process optimisation. Lactose is characterised in terms of particle size, PSD, morphological features, surface properties and polymorphic forms. Different grades of lactose have been shown to differ in their physico-chemical properties, which, in turn, result in varying dispersion properties and

aerosol performance. Moreover, the proportion of drug, coarse lactose and fine lactose and the blending method of a ternary DPI formulation have been shown to influence aerosol generation. It is clear that the interactions between all the parameters of a formulation need to be considered to produce DPI systems with the best performance. The number of grades of lactose has considerably increased this last decade, and attention must be paid when choosing a carrier for a new drug formulation. Fortunately, efforts are carried out by manufacturers to offer lactose grades for inhalation with controlled characteristics, such as PSD and shape as well as surface properties. Nevertheless, the physico-chemical characteristics of lactose may have a substantial effect on aerosol generation and drug delivery from DPI formulations; thus, care should be taken in establishing appropriate and highly specialised characterisation methods for selecting an appropriate grade of carrier. Moreover, the majority of results are obtained from powder mixtures prepared on a laboratory scale, and care should be taken by manufacturers in extending these conclusions to full-scale processing where mixing energies may be different.

To overcome the interparticulate forces between the drug and carrier and improve aerosol generation, optimising the surface properties of the carrier by surface treatment or addition of surface-controlled agents seems to be a promising approach. Nevertheless, despite all the work on these adhesive-cohesive forces, prediction of powder behaviour remains difficult because of the complexity and heterogeneity of the variables.

Aerosol generation of drugs depends upon a complex interaction between the device, the formulation and the patient, who controls the flow rate of inhaled air through the system. The drug formulation and inhaler device need to be optimised together to ensure reliable and effective drug delivery. Studying aerosol performance remains an active and important area for improving the delivery of various drugs for local or systemic action to the pulmonary tract and to the widest possible patient population.

Legend to figures

Figure 1. Illustration of the different causes of interactions between micronised particles (i.e., drug or fine excipients) and lactose carrier particles [113].

Figure 2. A. Schematic of regions on a carrier surface containing potential (1) high energy and (2) low energy 'active' sites. B. SEM of a crevice on a lactose carrier surface containing many micronised particles [40].

Figure 3. Steps in aerosol generation of the powder's static bed, including the main deagglomeration forces derived from the inspiratory flow during inhalation of a binary mixture of drug-lactose.

Figure 4. SEM images of a) Pharmatose 350, b) Pharmatose 350 processed by mechanofusion and c) Pharmatose 350 processed by mechanofusion with Mg st [87].

Figure 5. SEM images of inhalation grades lactoses: A. milled lactose monohydrate (Lactohale LH200) and B. sieved anhydrous lactose (Anhydrous 120MS) [182].

Figure 6. Mechanisms of drug dispersion for the 1. binary mixture, 2. ternary mixture, "Active site theory", and 3. ternary mixture, "Multiplets theory".

Figures

Figure 1

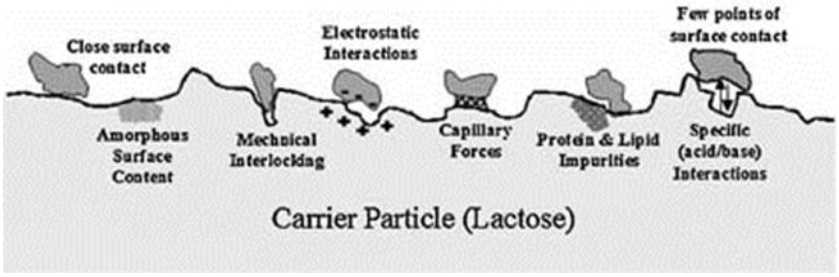


Figure 2

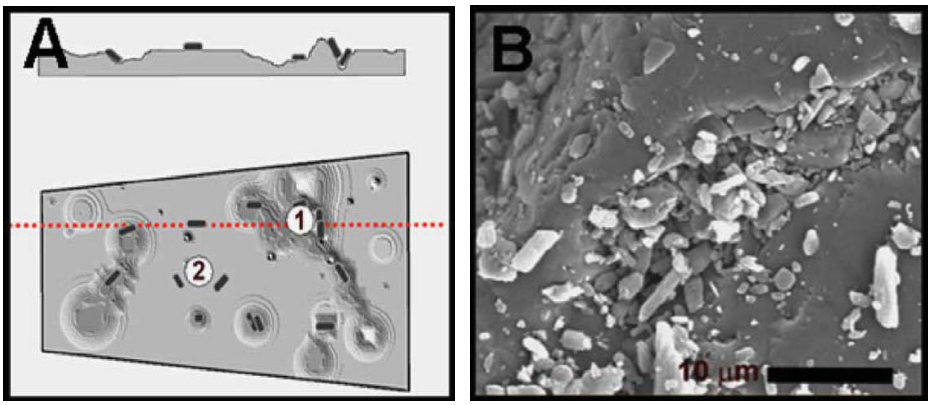


Figure 3

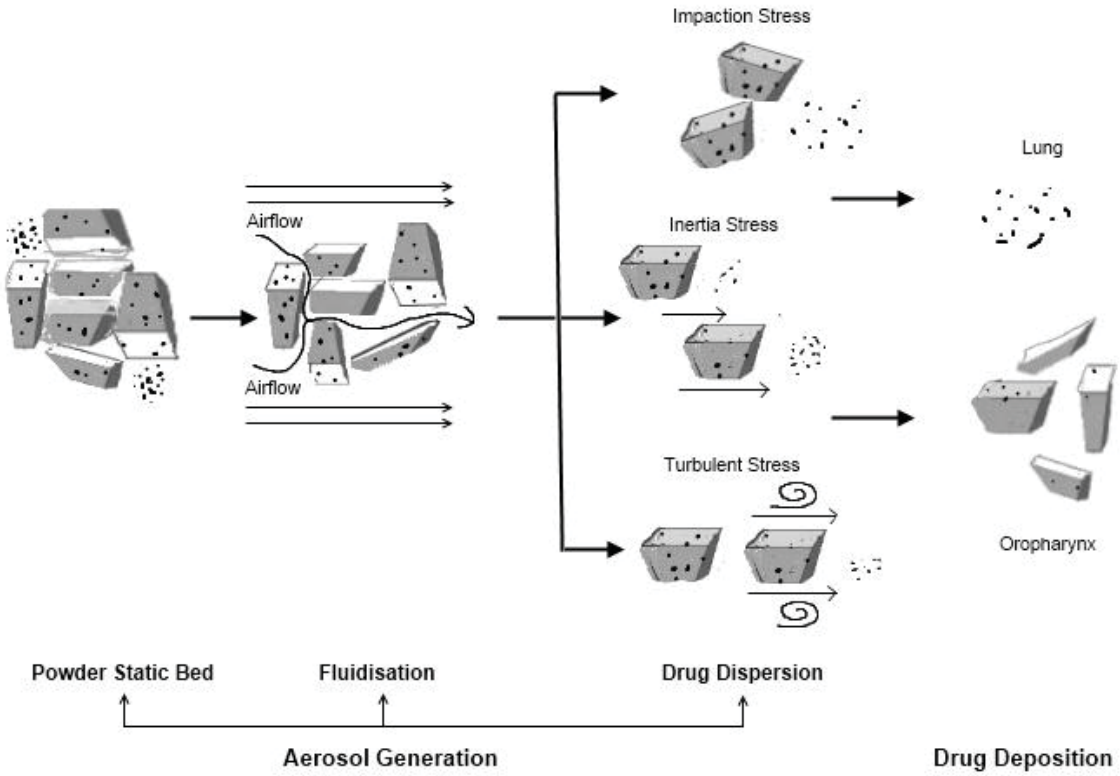


Figure 4

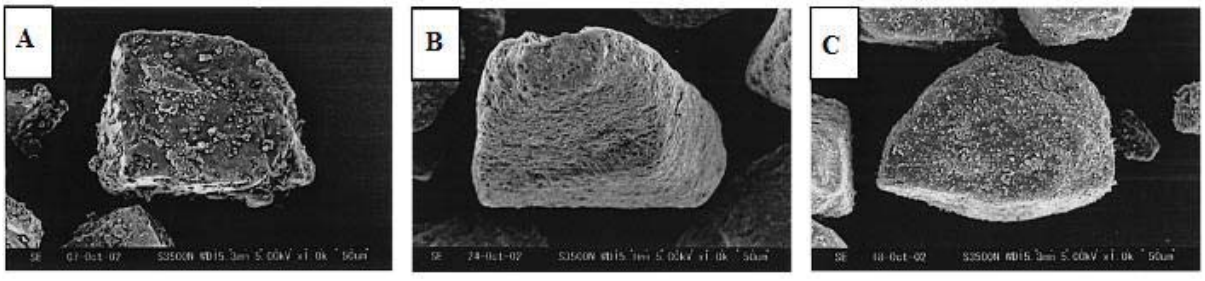


Figure 5

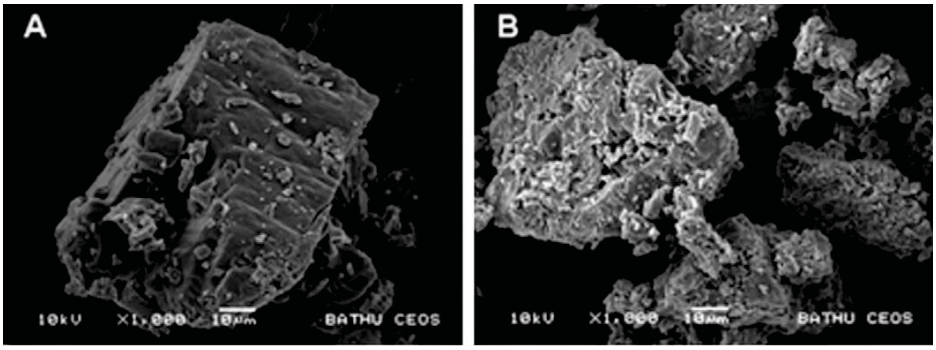
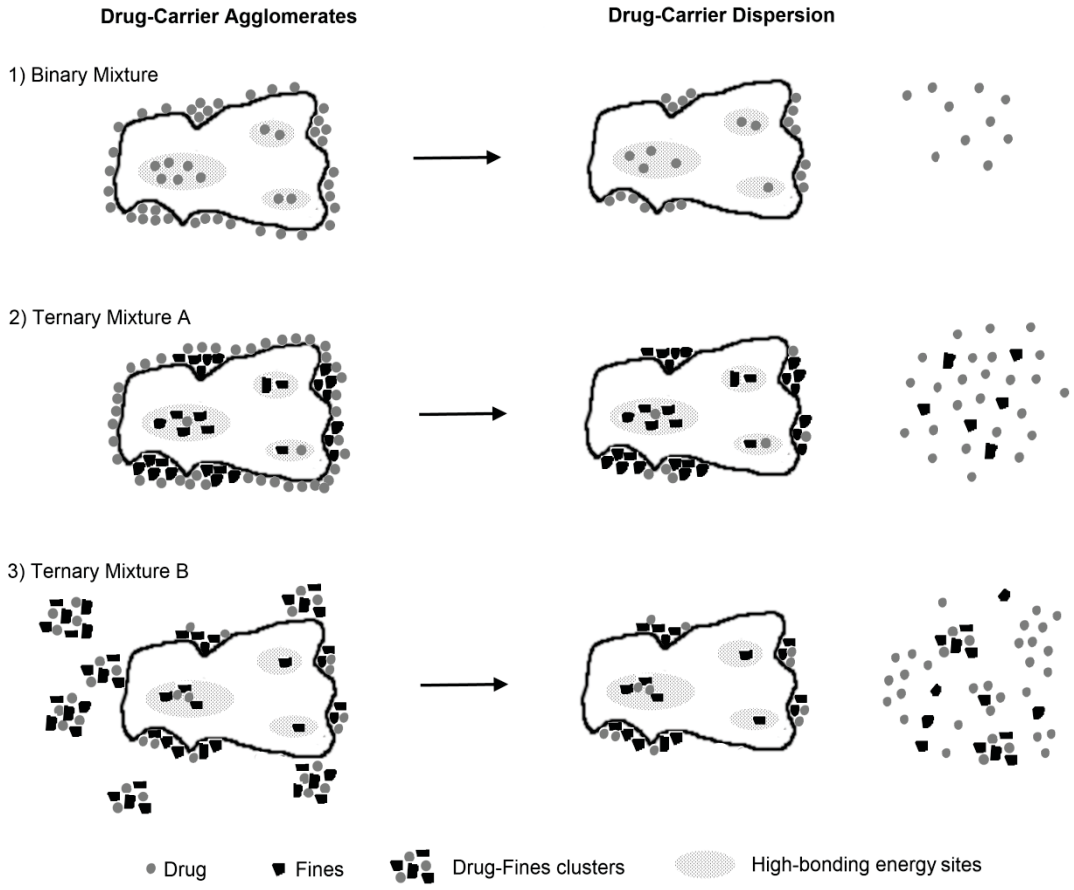


Figure 6



Tables

Table 1

Presentation of the main surface and bulk characteristics of the different available lactose for inhalation following their processing. The characteristics are relatively classified with + for the lowest and ++++ for the highest. NR means "non reported".

INHALATION GRADE LACTOSE									
Processing	**Roughness**	**Surface amorphous**	**Water uptake (at high RH)**	**Fine content (<10 µm)**	**Shape**	**Size d(0.5)**	**PSD**	**Carr's Index %**	**Examples of commercialy available lactoses**
LACTOSE MONOHYDRATE									
Sieved	+	+	+	+	tomahawk	~ 60	narrow/broad	21-25 / 16-20	Respitose SV003 [1], Inhalac 250 [2], Lacto-Sphere MM50 [3]
						~ 100	narrow	16-20	Inhalac 230 [2], Lactose MH Inhalation 120MS [4], Respitose SV010 [1]
						~ 130	narrow	11-15	Inhalac 120 [2], Lactohale LH 100 [5]
						200-220	narrow	1-15	Lacto-Sphere MM250 [3], Inhalac 70 [2]
Milled	++	++	+	++ (>10%)	tomahawk	50-100			Lactohale LH 200 [5], Lactose MH Inhalation 120M [4]
						50-60	broad	32-37	Respitose ML001 [1], Lactose MH Inhalation 80M [4]
						35-45			Lactose MH Inhalation 40M [4]
						17	narrow	>38	Respitose ML006 [1]
Micronized	++	+++	+	++++ (>90%)	tomahawk	< 5 µm	narrow	>38	Lactohale LH 300 [5], Lacto-Sphere MM3 [3], Respitose MC [1*]
Spray-dried	variable	++++ (from solution)	NR	NR	spherical	variable	narrow	NR	Respitose SD [1*]
Granulated						variable	variable	NR	Respitose GR [1*]
ANHYDROUS LACTOSE									
Sieved	+++	+	++	++	rounded	75-100	NR	21-25	Lactose AN NF Inhalation 120MS [4], Respitose AN [1*]
Milled	++++	++	++	+++	rounded	< 25µm	NR	NR	Lactose AN NF Inhalation 40M [4], Respitose AN [1*]

[1] DMV-Fonterra, [2] Meggle, [3] Micro-Sphere, [4] Sheffield, [5] Domo, * customized product

Table 2

Brief description of processing, resulting characteristics of surface modified or engineered lactose monohydrate carrier with the main improvements and potential limitations.

SURFACE MODIFIED OR ENGINEERED LACTOSE MONOHYDRATE CARRIER				
Processing	**Modifications**	**Improvement**	**Potential limitations**	**References**
Mechanofusion (rotor-type mixer) + Mg st or sucrose stearate	higher and homognenous surface energy , smoother and hydrophobic surface and rounder shape	higher dispersibility (higher FPF, lower ED) with fewer drug agglomerates	not approved agents, modified drug-carrier interaction, stability of blend	[87, 88]
Wet-smoothing (high shear mixer) using solvent with Mg st	smoother and hydrophobic surface, smaller surface area and surface energy, lower fine content, rounder shape	higher powder packing, flow properties and higher FPD, lower separation energy	modified drug-carrier interaction, stability of blend	[94, 95]
Surface erosion (high-speed elliptical-rotor-type mixer)	smoother surface, smaller specific surface area, more spherical shape and high shear force applied on surface	higher dispersibility (higher FPF, lower ED)	amorphous surface, stability of blend	[225]
Surface dissolution (temperature)	smoother surface, lower fines lactose content and similar flow properties and surface area	higher FPF, similar ED by reducing the number and the rank of 'active' sites	amorphous surface	[90, 226]
Surface dissolution (aqueous-ethanol solution at 70%)	lower macroscopic asperities decrease and higher microscopic asperities	higher dispersibility (higher FPF) by lower macroscopic and higher microscopic roughness	amorphous surface, stability of blend	[91]
Mg st coating (high-speed elliptical-rotor-type mixer)	smoother and hydrophobic surface and smaller surface area	Higher dispersibility (higher FPF) even at high RH	modified drug-carrier interaction, stability of blend	[227]
Mg st coating (Vortex mixing)	smoother and hydrophobic surface and smaller surface area	higher dispersibility (higher FPF, lower ED)	modified drug-carrier interaction, stability of blend	[227]
HPMC coating (fluidized bed)	smoother surface, smaller surface area and more spherical shape	higher dispersibility (higher FPF, lower ED)	HPMC not approved, stability of blend	[93]
Recrystallization from carbopol gel	smoother surface, more regular shape, narrower PSD	better flowability, higher and more reproductible ED and FPF		[85, 228]
Lactose composite (sub units fused)	homogeneous roughness surface, decreased contact area between drug and carrier	lower adhesion forces, no difference in ED, higher FPD	amorphous surface	[86]

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