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Review

Formulation strategy and use of excipients in pulmonary drug delivery

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ABSTRACT

Pulmonary administration of drugs presents several advantages in the treatment of many diseases. Considering local and systemic delivery, drug inhalation enables a rapid and predictable onset of action and induces fewer side effects than other routes of administration. Three main inhalation systems have been developed for the aerosolization of drugs; namely, nebulizers, pressurized metered-dose inhalers (MDIs) and dry powder inhalers (DPIs). The latter are currently the most convenient alternative as they are breath-actuated and do not require the use of any propellants. The deposition site in the respiratory tract and the efficiency of inhaled aerosols are critically influenced by the aerodynamic diameter, size distribution, shape and density of particles. In the case of DPIs, since micronized particles are generally very cohesive and exhibit poor flow properties, drug particles are usually blended with coarse and fine carrier particles. This increases particle aerodynamic behavior and flow properties of the drugs and ensures accurate dosage of active ingredients. At present, particles with controlled properties are obtained by milling, spray drying or supercritical fluid techniques. Several excipients such as sugars, lipids, amino acids, surfactants, polymers and absorption enhancers have been tested for their efficacy in improving drug pulmonary administration. The purpose of this article is to describe various observations that have been made in the field of inhalation product development, especially for the dry powder inhalation formulation, and to review the use of various additives, their effectiveness and their potential toxicity for pulmonary administration.

Contents

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1. Introduction

Local delivery of medication to the lungs is highly desirable, especially in patients with specific pulmonary diseases such as cystic fibrosis, asthma, chronic pulmonary infections or lung cancer. The principal advantages include reduced systemic side effects and higher doses of the applicable medication at the site of drug action. Although simple inhalation devices and aerosols containing various drugs have been used since the early 19th century for the treatment of respiratory disorders, a recent interest has arisen in systemic drug delivery via the pulmonary route. Interest in this approach has been stimulated by the potential utility of the lung as a portal for the entry of peptides and proteins. In fact, the lungs are an efficient port of entry for drugs to the bloodstream due to the large surface area available for absorption ($\sim$100 m^2), the very thin absorption membrane (0.1–0.2 μm) and the elevated blood flow (5 l/min), which rapidly distributes molecules throughout the body. Moreover, the lungs exhibit relatively low local metabolic activity, and unlike the oral route of drug administration, pulmonary inhalation is not subject to first pass metabolism (Adjei and Gupta, 1997).

Inhaled drug delivery devices can be divided into three principal categories: nebulizers, pressurized metered-dose inhalers (MDIs) and dry powder inhalers (DPIs); each class presents unique strengths and weaknesses. A good delivery device has to generate an aerosol of suitable size, ideally in the range of 0.5–5 μm, and provide reproducible drug dosing. It must also protect the physical and chemical stability of the drug formulation. Moreover, the ideal inhalation system must be a simple, convenient, inexpensive and portable device.

Nebulizers were the first devices developed for inhalation therapy market. Nevertheless, nebulization has disadvantages such as low efficiency, poor reproducibility and great variability. Moreover, aerosol administration via nebulization is time consuming; approximately 30 min if set-up, drug administration and cleaning are taken into account (Newhouse et al., 2003). Thus, further improvement in aerosol delivery systems with greater efficiency and portability and shorter administration time could improve patient quality of life and compliance.

Therefore, MDIs were developed and since the 1950s, they have been the mainstay of asthma inhalation therapy. Nevertheless, MDIs were recognized as not environmentally friendly and only a small fraction of the drug escaping the inhaler penetrates the patient's lungs due to a combination of high particle exit velocity and poor coordination between actuation and inhalation. The deposition of aerosolized drugs in the mouth and the oropharyngeal regions varies considerably according to the application technique, but losses using the pressurized devices are routinely greater than 70% and can exceed 90%. Particle losses that occur proximally to the lung are a long-documented problem that continues to compromise the effectiveness of current aerosol therapy protocols.

Given the problems encountered with nebulizers, alternative simple and small inhalers that do not use propellants were developed. DPI devices combine powder technology with device design to disperse dry particles as an aerosol in a patient's inspiratory airflow. Thus, they require little or no coordination of actuation and inhalation, which has frequently resulted in better lung delivery than was achieved with comparable MDIs. Interestingly, the introduction of "breath-actuated" MDI devices has provided a convenient and portable means of overcoming the major disadvantage of the need to coordinate the actuation with inhalation. Breath-actuated inhalers sense the patient's inhalation through the actuator and fire the inhaler automatically in synchrony. Examples are the Autohaler® (3M Pharmaceuticals, U.S.A.) or Easybreathe® (Norton Healthcare, U.K.).

Nevertheless, one of the major advantages of DPIs products is that they are propellant-free and are thus environmentally friendly. Moreover, DPIs are preferred for their stability and processing since they are typically formulated as one phase, solid-particle blends. DPIs 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 (Atkins, 2005). Today, there are essentially two types of DPIs: those in which the drug is packaged in individual doses (capsules) (single dose or unit-dose devices) and those that contain multiple doses in a foil–foil blister or a reservoir of drug from which the doses are metered (multi-unit and multi-dose devices).

A patient's DPI dose is dependent on four interrelated factors (Atkins, 2005): the properties of the drug formulation, particularly powder flow, particle size, shape and surface properties and drug carrier interaction; the performance of the inhaler device, including aerosol generation and delivery; correct inhalation technique for deposition in the lungs; and the inspiratory flow rate. Therefore, a balance among the design of an inhaler device, drug formulation and the inspiratory flow rate of the patient is required.

Since the creation of the first DPI, the Spinhaler®, device technology has continued to grow and many devices are now available on the market. Yet, the effect of a powder's interaction with the device on powder dispersion has generally been poorly understood. Recently, computational fluid dynamics has enhanced understanding of the impact of inhaler design on powder dispersion and deposition by demonstrating that small variations in device design can produce significant variations in performance (Coates et al., 2004).

Unfortunately, the choice of excipients used to enhance a powder's aerosol behavior is very limited for pulmonary applications. Alternative strategies involve the "engineering" of particles with controlled and suitable properties such as a narrow particle-size distribution and improved dispersibility. This paper provides a review of the different approaches to formulation strategies in the development of new inhalation products. As the use of DPIs is believed to grow, the authors will pay special attention to the different approaches to formulating these inhalation powders. Presently, over 20 DPI devices are available on the market and more than 25 are in development (Islam and Gladki, 2008). Since few excipients are authorized for the market, there is a special need for the development of modified particle and excipient properties to increase drug deposition in the lungs.

2. Particle aerodynamic diameter

Particle size is one of the most important design variables in an aerosol formulation, along with shape, density, electrical charge and hygroscopicity.

In fact, the three principal mechanisms of particle deposition in the respiratory tract rely on the size of the inhaled particles. Impaction is the inertial deposition of a particle onto an airway surface. It occurs principally at or near airway bifurcations, most commonly in extrathoracic and large conducting airways, where flow velocities are high and where rapid changes in the direction of bulk airflow often take place, generating considerable inertial forces. The probability of impaction increases with increasing air velocity, rate of breathing, particle size (>5 μm) and density. Gravitational sedimentation is an important mechanism for deposition of particles over 0.5 μm and below 5 μm in size in the small conducting airways where the air velocity is low. Deposition due to gravity increases with enlarging particle size and longer residence times but decreases as the breathing rate increases (Martonen and Yang, 1996). Submicron-sized particles (especially those less than 0.5 μm) acquire a random motion caused by the impact of surrounding air molecules. This Brownian motion may then result in particle deposition by diffusion, especially in small airways and alveoli, where bulk airflow is very low.

Therefore, to reach the lower respiratory tract and optimize pulmonary drug deposition, aerosols need to have aerodynamic diameters between 0.5 and 5 μm (Zanen et al., 1996). In other words, particles larger than 5 μm usually deposit in the oropharynx from which they are easily cleared. In contrast, particles smaller than 0.5 μm may not deposit at all because they move by Brownian motion and settle very slowly.

The aerodynamic diameter, which is defined as the diameter of a sphere with a unit density that has the same terminal settling velocity in still air as the particle in consideration (De Boer et al., 2002), is routinely measured by sizing techniques that are based on inertial impaction. The aerodynamic diameter (d_{ae}) is defined by the equation:

$$d_{ae} = d_{geo}\sqrt{\left(\frac{\rho_p}{\rho_0 \chi}\right)}$$

where d_{geo} is the geometric diameter, ρ_p and ρ_0 are the particle and unit densities, respectively, and χ is the dynamic shape factor.

Pharmaceutical powders are rarely spherical, and shape factors are dimensionless measures of the deviation from sphericity. The dynamic shape factor is the ratio of the actual resistance force experienced by a non-spherical falling particle to the resistance force experienced by a sphere having the same volume (Hinds, 1999). Consequently, the aerodynamic diameter can be decreased by decreasing the particle size, decreasing particle density, or increasing the dynamic shape factor.

Aerosol particle design therefore involves two basic strategies. Either particles are made with standard unit density with a geometric size in the 1–5 μm range, or they are created with a non-standard density with geometric sizes outside the standard range (Edwards and Dunbar, 2002). As an example, large porous particles exhibiting a high respirable fraction with mean geometric diameters ranging between 3 and 15 μm and tap densities between 0.04 and 0.6 g/cm^3 have been produced (Vanbever et al., 1999).

3. Forces of interaction

As particle-size distribution affects a drug's deposition in the lungs, separation of the small solid particles used in DPIs is the most important performance characteristic for effective aerosol generation. One way to improve the flow properties of a micron-sized drug is through the addition of carrier particles. To separate particles, the specific force of interaction must be overcome. There are four major forces of interaction between particles: mechanical interlocking due to surface asperities, capillary forces from the presence of water, electrostatic arising from the insulating nature of the material and van der Waals forces from the fundamental electromagnetic nature of matter (Telko and Hickey, 2005).

On a large scale, physical interactions are barriers to aerosol generation. Mechanical interlocking due to surface features or roughness is a prominent mechanism that prevents particle dispersion. These forces are related to the diameter of the pores between particles and interfacial tension due to hydrogen bonding of water. Controlling moisture content aids in reducing capillary forces, but care must be taken to avoid increasing the surface charge of the particles. Because most pharmaceutical powders are poor conductors, electrostatic charge plays a role in their dispersion. The magnitude of electrostatic forces is reciprocally, but not linearly, related to capillary forces (Telko and Hickey, 2005).

Capillary forces arise from the dynamic condensation of water molecules onto particle surfaces. If the amount of condensed water is sufficient, a meniscus is formed between contact points of adjacent surfaces as liquid is drawn by capillary action, inducing an attractive force (Begat et al., 2004b). In fact, at low relative humidity (RH), a crystalline material only adsorbs water onto its surface and moisture uptake is a function of the specific surface area of the material and the RH of the environment. As the RH increases, a complete monolayer of water molecules forms on the surface of the crystalline solid particles (Hancock and Shamblin, 1998). It has long been recognized that water condensation on the surface of the drug and/or carrier particles markedly increases the magnitude of cohesive forces (between micronized powder particles) and adhesive forces (between drug and carrier particles) (Price et al., 2002; Begat et al., 2004a). Hygroscopic drugs present a greater risk of physical and chemical instability. Moisture uptake and loss due to changes in RH can result in local dissolution and recrystallization, leading to irreversible aggregation though solid bridge formation, which can adversely affect aerosol generation and lung deposition (Hickey et al., 1990).

Because the adhesion of drug to carrier is a surface phenomenon, morphological differences may have a significant effect on the ability of the air flow to overcome these adhesive forces and to generate well-dispersed aerosol particles. Indeed, surface area is not solely determined by particle size and shape but also by surface morphology: corrugated particles have more surface area than smooth particles that occupy the same volume. Moreover, variation in surface roughness will alter the contact area between contiguous surfaces and thus alter the total van der Waal's force of interaction and the propensity for surface charging or the degree of water condensation (Young et al., 2007). It has been reported in several previous studies that increasing the surface smoothness of the carrier particles increases the fine particle fraction (FPF) of the drug (Zeng et al., 2000; Young et al., 2002).

4. Particle engineering

Given that the number of excipients allowed in pulmonary drug delivery for improving the aerosol behavior of formulations is very restricted, superior delivery efficiency may be achieved by developing optimized particulate formulations. This alternative strategy, which is synonymous with the controlled production of drug particles, either in pure physical forms or with carriers as composite materials, of optimized size, morphology and structure, is broadly referred to as "particle engineering". The main goal of particle engineering is to incorporate desirable attributes, such as narrow particle-size distribution, improved dispersibility, enhanced drug stability, optimized bioavailability, sustained release and/or precise targeting, into particles while taking into account the specifics of inhaler design and drug delivery requirements. The complex nature of interparticulate interactions on the lower micron-sized scale, together with the extensive application of dry powder formulations and the specifics of aerodynamic behavior within the inhaler devices and respiratory system, have rendered the development of such dosage form one of the most challenging areas of inhalation pharmaceutics. There are several options for reducing the particle size. Typically, the first size reduction technique is milling.

4.1. Milling

There are many different mills, but only a few are able to mill powder to the required particle size range of 1–5 μm. The two main types of mills used are ball mills and fluid-energy mills, such as the jet mill, which is a well-established and well-validated technique used to manufacture dry powders for inhalation.

Jet milling is the most useful technique; it reduces particle size via high velocity particle–particle collisions. Unmilled particles are introduced into the milling chamber. Air or nitrogen, fed through nozzles at high pressure, accelerates the solid particles to sonic velocities. The particles collide and fracture. While flying around the mill, larger particles are subjected to higher centrifu-

gal forces and are forced to the outer perimeter of the chamber. Small particles exit the mill through the central discharge stream. Depending on the pressure and powder feed rate, particles with diameters down to approximately 1 μm can be produced (Telko and Hickey, 2005). For example, clarithromycin exhibits a median size of 1.9 ± 0.1 μm and lactose a $D(0.5)$ of 2.5 ± 0.1 μm after treatment. In fact, most micronized drugs sold by manufacturers on the market are produced by jet milling. Nevertheless, 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.

A ball mill is essentially a rotating cylinder loaded with drug and "milling media" (i.e., balls that grind the drug between each other as they tumble inside the mill). The size and material of the milling media can vary. Ball milling is very slow and the process is poorly scalable, which is why tumbling-ball mills are only used for laboratory-scale (Telko and Hickey, 2005).

However, strong mechanical processing, such as milling, has been shown to affect the crystallinity of the material. Amorphous regions can be generated at the surface of the fractures' crystals (Malcolmson and Embleton, 1998). Being thermodynamically unstable, such amorphous domains will undergo recrystallization leading to crystal growth on the milled particle surface and formation of solid bridges between the particles. The material is also prone to chemical decomposition and water sorption. The micronization process leads to small, irregularly shaped flat particles, and extensive flat surfaces promote large contact areas, resulting in increased adhesion between the particles. Micronized powders with high energetic surfaces show poor flow properties (Feeley et al., 1998); smaller particles yield stronger cohesive forces. Moreover, this process provides only a limited opportunity for control over important particle characteristics such as size, shape and morphology. It may not be suitable for fragile molecules and more complex engineered structures, such as porous/hollow particles and nanoaggregates, or for surface-modified, coated or encapsulated materials.

Other techniques for making micron-sized particles involve spray drying and supercritical fluid (SCF). These noteworthy approaches allow the production of particles with controlled and appropriate size and shape. These techniques are distinctly different from milling in that the particles are built up (i.e., particle size is increased) whereas particle size is decreased during milling.

4.2. Spray drying

Spray drying is a one-step process that converts a liquid feed to a dried particulate. The feed can be a solution, a coarse or fine suspension or a colloidal dispersion (e.g., emulsion, liposome, etc.), which is first atomized to a spray form that is put immediately into thermal contact with a hot gas, resulting in the rapid evaporation of the droplets to form dried solid particles. The dried particles are then separated from the gas by means of a cyclone, electrostatic precipitator or bag filter.

The three fundamental operations of the spray drying process are atomization, drying and separation. The design may be "open cycle", where the drying gas (usually air) is not recirculated and is vented to the atmosphere. When spray drying from organic feeds, a "closed cycle" layout is more suitable than an open one since the risk of flammability and explosion is higher in the latter when organic solvents are heated in the presence of oxygen. In the closed cycle design, the heated gas, which is usually nitrogen with less than 5% oxygen, is recirculated, and a condenser is employed to convert organic vapors to the liquid state (Sacchetti and Van Oort, 1996).

Atomization is the process whereby a liquid is broken up into a collection of droplets. The principal atomization methods are centrifugal, pneumatic, ultrasonic, electrostatic and effervescent. Pneumatic, or twin-fluid, atomization is suited for generating particles in the size range of inhalation products and for laboratory-scale research. In this process, the liquid feed is pumped at low flow rates to the orifice of the nozzle where the liquid comes into contact with a gas under pressure at high velocity, which creates the spray.

The nozzle design and the type of atomization can affect droplet size and provide control over particle-size distribution. In the "co-current" method, the nozzle is positioned at the top of the drying chamber and the spray enters in the same direction as the gas flow. In this approach, the droplets are subjected to the highest temperatures in their most moist state followed by rapid evaporation and cooling. This method is particularly suitable for heat-labile materials and compounds with low melting points or glass transition temperatures as the drying time ranges from 100 milliseconds to seconds (Johnson, 1997).

Once the liquid is atomized, droplets dry to solid particles through intimate contact with heated gas in the drying chamber. The important drying variables are inlet and outlet temperatures, the drying gas medium, gas humidity, gas flow rate and residence time, which all together 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 vapor must be removed to reduce the residual solvent to pharmaceutically acceptable levels.

For laboratory-scale work, the most widely used powder separation technique is the cyclone. In this technique, a centrifugal force is applied to spray-dried particles, which then impact the walls and are removed from the upward-flowing drying gas by collection in a bottom vessel. The collection efficiency can be enhanced by increasing gas inlet velocity and increasing mass loading (Sacchetti and Van Oort, 1996).

Compared to milling, spray drying produces more spherical particles. Such particles are characterized by a lower area of contact and a smaller, more homogeneous particle-size distribution that result in a higher respirable fraction than mechanically micronized drugs (Steckel and Brandes, 2004). Nevertheless, particles from spray drying processes are not always spherical and may have convoluted surfaces, asperities, holes and voids. In fact, the shape is influenced by the drying rate, the surface tension and viscosity of the liquid (Johnson, 1997). One of the principal purposes of aerosolizing spray-dried powders is to achieve particle diameters of several micrometers with a narrow particle-size distribution. This ensures, assuming an appropriate aerodynamic diameter, a maximum deposition of the embedded drug in the tracheo-bronchial and deep alveoli regions at normal inhalation rates.

It is also important to note that spray-dried particles from solutions are mostly amorphous, but to maintain the crystalline state of the drug, suspensions can be processed.

The principal advantages of spray drying with respect to pulmonary drug delivery are the ability to manipulate and control a variety of parameters such as solvent composition, solute concentration, solution and gas feed rate, temperature and relative humidity, droplet size, etc. This allows optimization of particle characteristics such as size, size distribution, shape, morphology and density, in addition to macroscopic powder properties such as bulk density, flowability and dispersibility (Sacchetti and Van Oort, 1996). This battery of controllable parameters offered by spray drying is a great advantage over jet milling. Moreover, spray dryers are available at different production scales and industrial scale up is easy.

Spray drying is a commonly used technique in pharmaceutical industries for preparing dry powders for inhalation carrying vari-

ous drugs such as budesonide, salbutamol, beclomethasone (Sebti and Amighi, 2006; Learoyd et al., 2009) or peptides and proteins such as deoxyribonuclease and insulin (Klinger et al., 2009; Zijlstra et al., 2009). For example, it is interesting to note that spray drying markedly enhanced the FPF of tobramycin from approximately $36 \pm 6\%$ in its raw state to approximately $49.3 \pm 0.8\%$ after spray drying in suspension (Pilcer et al., 2009a). Moreover, spray-dried drugs exhibited a narrow particle-size distribution with a median diameter of approximately $1.32 \pm 0.09\,\mu m$. However, degradation during the spray drying process may be a problem for some macromolecules as a result of a number of factors such as thermal stress during droplet drying, high shear stress in the nozzle and peptide/protein adsorption at the greatly expanded liquid/air interface of the spray solution.

4.3. Spray-freeze-drying

This technique involves spraying a solution containing the drug into a vessel containing a cryogenic liquid such as nitrogen, oxygen or argon. Since the normal boiling point for such a liquid is very low, the droplets are quickly frozen. Lyophilizing these frozen droplets results in porous spherical particles suitable for inhalation. Spray-freeze-dried particles can be engineered to the desired respiratory range (below 5 μm) or even down to nano-scale. Spray-freeze drying is also used to produce liposomial formulations for MDIs and liquid formulations.

Unlike spray drying, spray-freeze-drying is conducted at sub-ambient temperature, and has, therefore, been used to formulate a significant number of thermolabile and highly potent therapeutic proteins/peptides into dry powder inhalation products, including recombinant-derived humanized anti-IgE monoclonal antibody, recombinant human deoxyribonucleases, insulin, cetrorelix acetate (Chow et al., 2007).

Although this technique has been used for producing protein particles, it is faced with the limitations of stresses associated with freezing and drying, which may cause irreversible damage to the protein. This is manifested as structural denaturation, aggregation and loss of biological activity upon rehydration. Similar to spray drying, loss of stability due to unfolding and aggregation remains a major challenge. Further possible limitations to this technique include the fact that it is time consuming, the nature of the process has safety issues involved with spraying into cryogenic fluids and it is expensive.

4.4. Supercritical fluids

Supercritical fluids (SCF) are defined as compressed gases or liquids above their critical pressures and temperatures, which 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 bar, and its low cost and non-toxicity, is the most widely used solvent in many SCF processes (Johnson, 1997). Its critical temperature makes SCF suitable for processing heat-labile solutes at conditions close to room temperature.

The three main SCF processes are precipitation from supercritical solutions composed of supercritical fluid and solutions (RESS), precipitation from gas saturated solutions (PGSS) and precipitation from saturated solutions using supercritical fluid as antisolvent (SAS).

The first method (RESS) involves dissolving or solubilising the drug in a SCF followed by rapid expansion of the fluid solution across a heated orifice to cause a reduction in the density of the solution, thereby decreasing the solvation power of the fluid which leads to precipitation of the drug. In the second method (PGSS) the SCF is dissolved in molten solute and the resulting supercritical solution fed via an orifice into a chamber to allow a rapid expansion under ambient conditions. Nevertheless, scale up of these techniques is limited by particle aggregation and nozzle blockage caused expansion cooling. It is also limited to molecules that are soluble in carbon dioxide. Therefore, the third method (SAS) which relies on the capacity of an SCF to act as an antisolvent is the most common method and different variants of the technique have been developed. The high solubility of carbon dioxide in organic solvents leads to volume expansion when the fluids make contact. This leads to reduction in solvent density and parallel fall in solvation capacity. Such reduction causes increased level of supersaturation, solute nucleation and particle formation. In this process, the SCF is added to the particle formation vessel that contains the drug solution of interest. The drug precipitates during the dissolution of the SCF in the solvent. The SCF is generally introduced through the bottom of the vessel and bubbled through the solution to achieve better mixing of the solvent and antisolvent. Once the drug has precipitated out, the solvent–SCF can be removed.

The solubility of a drug powder in supercritical fluid will depend on the density of the fluid, the drug's chemical structure and the supercritical fluid–drug contact time. The morphology and size distribution of the particles formed are dependent on the pre-expansion concentration of the solute in supercritical fluid and the expansion conditions (e.g. temperature, pressure). The higher is the pre-expansion concentration, the narrower is the particle-size distribution. The expansion conditions depend on the nozzle temperature, geometry and size. The nozzle has to be maintained at a suitable pre-expansion temperature to prevent the premature precipitation of the drug.

All three techniques allow production of inhalable particles with a significant increase in FPF compared to jet-milled products (Shekunov et al., 2004; Chow et al., 2007). Anti-asthmatic drugs such as budesonide, fluticasone, salmeterol, albuterol, terbutaline, fenoterol and peptides such as insulin and cyclosporine have been successfully produced by SCF technology (Rehman et al., 2004; Shekunov et al., 2004; Lobo et al., 2005; Amidi et al., 2008). For instance, cyclosporine was precipitated as microspheres as small as 150 nm and easily dispersible budesonide crystals with aerodynamic diameter between 1 and 2 μm were obtained for dry powder formulations.

Fine drug particles produced via SCF precipitation are less charged than those produced by mechanical means, which allows them to flow more freely and to be more easily dispersed following discharge from a DPI. Moreover, SCF processes permit the production of respirable drug particles that are intrinsically more uniform in terms of crystallinity, morphology, particle-size distribution and shape than those produced via jet milling (Malcolmson and Embleton, 1998). Particle engineering with supercritical fluids is the subject of intense research in the pharmaceutical industry (Schiavone et al., 2004; Lobo et al., 2005). Despite its potential, SCF is still an emerging technology that is not much exploited in DPI products; concern over the potential denaturing effects of the solvents/antisolvents used in this process is a drawback.

It is important to consider the effect each technique has on the drug. Spray drying and supercritical fluid methods offer more flexibility and the possibility of control over morphology and size, but they occasionally yield amorphous material or undesired polymorphs. Milling remains the process of choice for micronizing drugs because it is simpler, more predictable, easier to scale up and less expensive. However, spray drying, supercritical fluid processing and a few other techniques remain alternatives for the formulator to consider when milling does not produce the desired results. Interestingly, these techniques may also be used to produce coated particles or matricial formulations entrapping the active drug.

5. Formulations of inhalation products with excipients

In general, excipients are used to enhance the physical or chemical stability of the active pharmaceutical ingredient, its mechanical properties and/or its pharmaceutical properties such as dissolution and permeation. In fact, excipients are inactive ingredients that are intentionally added to therapeutic products but are not intended to exert therapeutic effects at the intended dosage, although they may act to improve product delivery or efficacy. The Food and Drug Administration (FDA, 2009) favors the use of commercially established excipients as well as "generally recognized as safe" (GRAS) substances. It should be noted that the current excipients approved for respiratory drug delivery are very limited in number (Tables 1–3).

Unfortunately, published literature guidance about the safety evaluation of pharmaceutical excipients used in inhalation and about application for marketing authorization is very limited (Baldrick, 2000). Despite the lack of regulatory guidance, the development of new excipients or the improvement of existing ones has been expanding in recent years. Some guidance describes the types of toxicity data used to determine whether a potential new excipient is safe for use in human pharmaceuticals (ICH, M3, S3A and S7A). This requires evaluation of a safety database. Existing human data for some excipients can substitute for certain non-clinical safety data, and an excipient with documented prior human exposure under circumstances relevant to the proposed use may not require evaluation with the full battery of toxicology studies outlined in the guidance.

Otherwise, a complete toxicological evaluation of the excipient alone and bridging studies in animals with the new complete formulation are generally sufficient for regulatory approval for a new excipient with unknown inhalation toxicology potential. However, it is surprising how little useful toxicology data is available for many well-known materials.

The strategies recommended (Baldrick, 2000, 2008) to support marketing of new excipients may differ slightly for short- or long-term intended use (Fig. 1). In general, the goals of a non-clinical safety evaluation include a characterization of toxic effects with respect to target organs, dose dependence, relationship to exposure and, when appropriate, potential reversibility. New potential

Table 1
Example of marketed nebulization products.

Products	Drugs	Additives
ATROVENT DUOVENT (BOEHRINGER INGELHEIM)	Atrovent: Ipratropium Br 0.25 mg or 0.5 mg/2 ml Duovent: Ipratropium Br 0.5 mg + Fenoterol 1.25 mg/2 ml	NaCl HCl Aqua
BISOLVON (BOEHRINGER INGELHEIM	Bromhexine HCl 2 mg/ml	Tartaric acid Methyl parahydroxybenzoate Aqua
FLIXOTIDE (GLAXOSMITHKLINE)	Fluticasone propionate 2mg/2ml	Polysorbate 20 Sorbitane laurate Dihydrated monosodic phosphate Anhydrous disodic phosphate NaCl Aqua
FLUIMUCIL ANTIBIOTIC (ZAMBON)	thiamphénicol glycinate acétylcystéinate 405 mg/ml	Sodium Méthylparahydroxybenzoate Propylparahydroxybenzoate Aqua
LOMUDAL (SANOFI – AVENTIS)	Sodium cromoglycate 20 mg	Aqua
LYSOMUCIL 10% (ZAMBON)	Acetylcysteine 300 mg/3ml	EDTA NaOH at pH 6.5 Aqua
MISTRABON (UCB PHARMA)	Mesna 200 or 600 mg	EDTA NaOH at pH 7.5 Aqua
PULMICORT (ASTRAZENECA)	Budesonide 0.25 or 0.5 mg/ml	EDTA NaCl Polysorbate 80 Citric acid Na citrate Aqua
PULMOZYME (ROCHE)	Dornase alpha 2.5 mg/2.5 ml	NaCl CaCl Aqua
TOBI (SOLVAY PHARMA)	Tobramycin 300 mg/5ml	NaCl H_2SO_4 and NaOH ad pH 7 Aqua
VENTAVIS (SCHERING)	Iloprost 10 μg/ml	Trometamol Ethanol 96° NaCl HCl Aqua
VENTOLIN (GLAXOSMITHKLINE)	Salbutamol 5 mg/ml	Cl benzalkonium H_2SO_4

Table 2
Example of marketed MDI products.

Products	Drugs	Additives
AEROBID (FOREST)	Flunisolide 250 μg	Trichloromonofluoromethane Dichlorodifluoromethane Dichlorotetrafluoroethane Sorbitan trioleate Menthol
AIROMIR (UCB PHARMA)	Salbutamol 100 μg	Norflurane (HFA-134a) Oleic acid Ethanol
ATROVENT DUOVENT (BOEHRINGER INGELHEIM)	Ipratropium Br 20 μg Ipratropium Br 20 μg + Fenoterol 50 μg	Tetrafluoroéthane Citric acid Ethanol Aqua
BECLOJET (CHIESI SA)	Beclomethasone 250 μg	HFA-134a Ethanol Glycerol
COMBIVENT (BOEHRINGER INGELHEIM)	Ipratropium Br 20 μg Salbutamol 120 μg	Dichlorodifluoromethane Dichlorotetrafluoroethane Trichloromonofluoromethane Soya lecithin
DUO-MEDIHALER (3M PHARMACEUTICALS)	Isoproterenol 0.16 mg Hydrochloride ditartrate phenylephrine 0.24 mg	Dichlorodifluoromethane Dichlorotetrafluoroethane Trichloromonofluoromethane Cetylpyridinium chloride Sorbitan trioleate
FLIXOTIDE SERETIDE VENTOLIN (GLAXOSMITHKLINE)	Flixotide: Fluticasone propionate 50 or 250 μg Seretide: Salmeterol 25 μg + Fluticasone propionate 50, 125 or 250 μg Ventolin: Salbutamol 100 μg	Norflurane
LOMUDAL (SANOFI – AVENTIS)	Na cromoglycate 5 mg	Heptafluoropropane: HFA-227 Polyvinylpyrrolidone K30 Polyethylene glycol 600
PULMICORT (ASTRAZENECA)	Budesonide 50 or 200 μg	Norflurane Sorbitan. Trioleate Magnesium stearate
SEREVENT (GLAXOSMITHKLINE)	Salmeterol 25 μg	Trichlorofluorométhane Dichlorodifluorométhane Lecithin

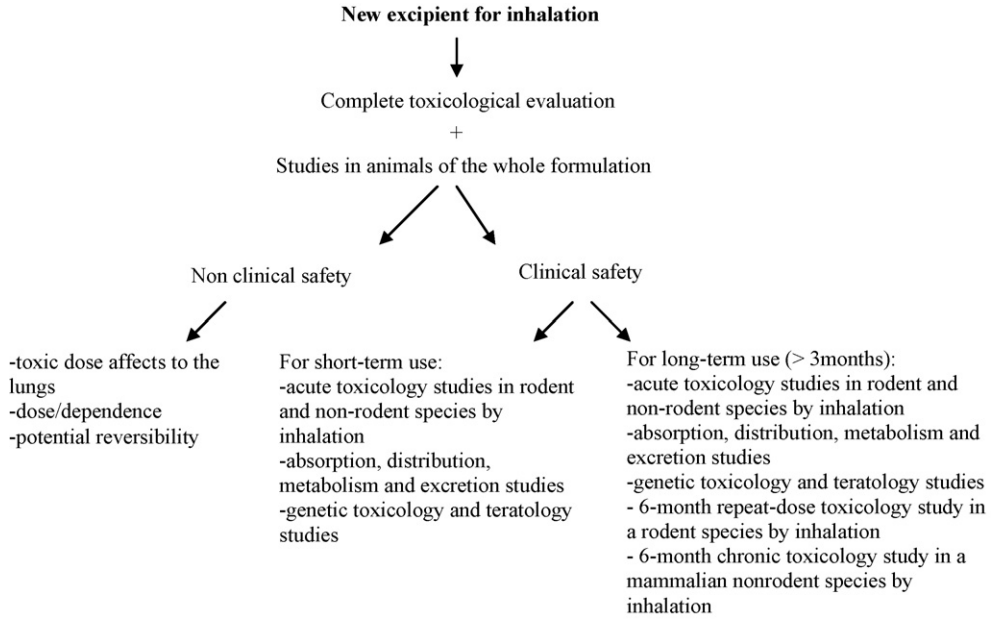

Fig. 1. Summary of the safety evaluation of a new excipient for inhalation use.

Table 3
List of accepted or interesting additives for DPI formulations.

Excipients	Description	Status
Sugars	Coarse/fine carrier	
Lactose		Approved and commonly used
Glucose		Approved (Bronchodual®)
Mannitol		Approved (Exubera®)
Trehalose		Promising alternative (Mao and Blair, 2004)
Hydrophobic additives	Protection for drug moisture	
Mg stearate		Approved (SkyeProtect™)
Lipids	Used in liposomes, matrix, coating	
DPPC, DSPC, DMPC, cholesterol		Biocompatible and biodegradable, very interesting excipients (Myers et al., 1993; Sebti and Amighi, 2006; Chono et al., 2009)
Amino acids	Improved aerosol efficiency	
Leucine, trileucine		Endogenous substance but no data on lung toxicity (Rabbani and Seville, 2004; Lechuga-Ballesteros et al., 2008)
Surfactants	Production of light and porous particles	
Poloxamer		May not be pro-inflammatory at low dose (Steckel and Brandes, 2004; Vaughn et al., 2007)
Bile salts		Endogenous substances, May be accepted but at low dose (2–5%, w/w) (Yamamoto et al., 1997; Johannson et al., 2002; Pilcer et al., 2009b)
Absorption enhancers	Absorption for proteins & peptides	
Hydroxypropylated-β-CD, natural γ-CD		Promising results (Hussain et al., 2004; Salem et al., 2009)
Bile salts		Promising results but toxic in chronic use (Yamamoto et al., 1997; Johannson et al., 2002)
Chitosan, trimethylchitosan		Pro-inflammatory effect observed (Huang et al., 2005; Grenha et al., 2008)
Biodegradable polymers	Used in sustained release formulations	
PLGA		Immunogenicity effect observed (Zeng et al., 1995; Dailey et al., 2006)

excipients must be evaluated for pharmacological activity using a battery of standard tests described in ICH guidance for industry (ICH, M3, S3A and S7A). It is recommended to perform acute toxicology studies in both a rodent species and a mammalian non-rodent species by the route of administration intended for clinical use. It is not necessary to determine the LD_{50} of an excipient. It is recommended that the absorption, distribution, metabolism and excretion of the excipient be studied following administration; the reproductive toxicology of the excipient must also be evaluated, including assessment of its potential effect on fertility or early embryonic development to implantation and teratology. Reproductive studies must be conducted in both a rodent species and a mammalian non-rodent species, and attention must be paid to the excipient's effects on prenatal and postnatal development including maternal function. The most efficient way to address these different developmental landmarks is use of a single study rodent assay to assess all phases of reproductive toxicity in conjunction with a teratology study in a non-rodent species, provided that the available data predict that the excipient displays minimal toxicity. For long-term use, it is recommended that a 6-month repeat dose toxicology study be performed in a rodent species by the appropriate route. It is important that the study includes complete clinical pathology, histopathology and toxicokinetic analyses and that a chronic toxicology study be performed in a mammalian non-rodent species (FDA guidance).

A decision to develop new excipients must take into account the extra workload, time and cost involved. Indeed, drug companies have to assess carefully the benefits of using the new substance in light of possible regulatory delays/rejection. If necessary, the development of new excipients will be limited to physiological and biocompatible additives and otherwise to well-known additives used in other routes. Another alternative is to develop different grades of the same excipient with various properties such as for the lactose for example. In the near future, it is probable that new excipients will have to be developed to allow the delivery of new drugs such as peptides and proteins, monoclonal antibodies or genes. In the case of expensive drug formulations or new treatments for serious pathologies, the risk of rejection by regulatory authorities may decrease to promote research.

5.1. Excipients used in nebulization

There are two traditional devices used in nebulization: air-jet and ultrasonic nebulizers. For a typical jet nebulizer, compressed air passes through a narrow hole and entrains the drug solution from one or more capillaries mainly by momentum transfer. Alternatively, ultrasonic nebulizers use a high frequency vibrating plate to provide the energy needed to aerosolize the liquid. The frequency of the vibrating piezoelectric crystal determines the droplet size for a given solution. Approximately 70% of the particles produced present sizes of between 1 and 5 μm. Nevertheless, heat resulting from frictional forces induced by movement of the transducing crystal may be detrimental to thermolabile formulations.

Nebulizers have been used in inhalation therapy since the early 19th century. But nebulization has many well-documented disadvantages, including extended administration time, high cost, low efficiency, poor reproducibility and great variability, risk of bacterial contamination and constant cleaning requirements, and sometimes the need for bulky compressors or gas cylinders (Newhouse et al., 2003).

Marketed respiratory solutions are generally composed of drugs dissolved in aqueous, isotonic solvent systems that may con-

tain preservatives to reduce microbial growth (Table 1). In the past, inhaled antibiotic preparations were prepared from intravenous medications that were not originally intended for delivery to the airways. Several of these preparations contained preservatives, such as phenol and bisulfite, which can contribute to airway irritation, coughing and bronchoconstriction. In addition to an unpleasant taste, phenol is a neurotoxin and is listed by the National Institute for Occupational Safety and Health as being occupational exposure hazard. Nowadays, to enhance chemical stability, sodium bisulfite and ethylenediaminetetraacetic acid (EDTA) are authorized in various preparations. Nevertheless, they are both known to cause bronchoconstriction (Nikolaizik et al., 1996).

Sodium chloride (NaCl) and other salts are widely used to adjust the osmolarity of solutions to approximately 300 mosmol/l. Attention must also be paid to the pH of the formulation because, unlike the gastrointestinal tract, the lungs have limited buffering capacity. Therefore, HCl, NaOH, citric acid, phosphates and trometamol are commonly used to adjust the pH of the solution to neutrality.

Accepted surfactants such as polysorbates and sorbitanes are generally present to aid dispersion or dissolution of the drugs. A co-solvent such as ethanol may be used only in small amounts because of alcohols' propensity to irritate the lungs.

Finally, some formulations also contain preservatives such as methylparaben and propylparaben to ensure long-term conservation of the products.

5.2. Excipients used in MDIs

Asthma therapy has primarily employed the pressurized metered-dose inhaler. In an MDI, the drug is either suspended or dissolved in a propellant that is pressurized until it liquefies in a canister. The liquefied propellant serves both as a source of energy for expelling the formulation from the valve in the form of rapidly evaporating droplets and as a dispersion medium for the drug and other excipients. Despite the presence of gas propellant, the additives used in MDI formulations are similar to those used in nebulization (Table 2). Chlorofluorocarbon (CFC)-based MDIs generally contained a combination of a liquefied low boiling-point propellant, CFC 12 (dichlorodifluoromethane), and a liquefied higher boiling-point propellant like CFC 11 (trichlorofluoromethane) or CFC 114 (dichlorotetrafluoromethane). However, the success of CFC propellant-driven MDIs has been overshadowed by their contribution to ozone depletion in the upper atmosphere and concomitant health effects (Molina and Rowland, 1974). In fact, the international community agreed to phase out CFC propellants in 2000. Presently, the main emphasis in MDI development is on introduction of non-CFC propellants. But the non-CFC propellant hydrofluoroalkanes, HFA 134a (1,1,1,2-tetrafluoroethane) and HFA 227 (heptafluoropropane), which are now considered established excipients, have very different physical properties, and, in particular, compatibility problems with the valve components and extremely poor solvent properties. This feature is beneficial in preventing the dissolution of small drug particles, but it is also disadvantageous in that commonly used surface-active agents are almost totally insoluble and not able to provide any physical stabilization of drug particles in suspension. There have been numerous approaches taken to overcome the problems of drug–particle instability in HFA propellants, including addition of a co-solvent such as ethanol, developing new specific surface-active agents, reducing the interfacial tension by modifying the particle surface properties and particle engineering to produce a more HFA-compatible material (Smith, 2002). Accepted surfactants such as sorbitan trioleate (SPAN 85), oleic acid and soya lecithin, at levels between 0.1% and 2.0% (w/w), are typically present to aid dispersion of suspended drug particles or dissolution of a partially soluble drug and to lubricate the metering mechanism (Newman, 2005). Flavors and suspended sweeteners may be present to combat the unpleasant taste. To enhance chemical stability, antioxidants (ascorbic acid) or chelating agents (EDTA) may be added to the formulations (Dalby et al., 1996).

5.3. Excipients used in DPIs

As a result of the problems encountered with MDIs, the design and use of alternative inhalers that do not use propellants were developed. These devices combine powder technology with device design in order to disperse dry particles as an aerosol in the patient's inspiratory airflow. All DPIs have four basic features: a dose-metering mechanism, an aerosolization mechanism, a deaggregation mechanism, and an adaptor to direct the aerosol into the mouth.

Particle-size distribution affects a drug's deposition in the respiratory tract. However, smaller particles undergo stronger cohesive forces, resulting in increased adhesion between the particles leading to poor flow properties (Feeley et al., 1998). One way to improve the flow properties of a micron-sized drug is through the addition of excipients. In DPI, most of the formulations contain excipients as carrier particles. Usually, no more than a few milligrams of drug need to be delivered (e.g., corticosteroids for asthma therapy), and excipients provide bulk, which improves handling, dispensing and metering of the drug.

5.3.1. Blending

After drug and excipient(s) have individually been brought to their desired forms, they are combined in a blending process. The flow properties of the components of the powder blend will play an important role in the efficiency of blending and, ultimately, in aerosol dispersion. Blended formulations consist of small drug particles that are mixed with large excipient carrier particles (50–200 μm). It is a critical step in the manufacture of a DPI product; in fact, it is subject to substantial optimization work during development. When mixing powders with different properties, particle sizes and ratios, as is the case with DPI formulations, inadequate mixing can cause poor dose uniformity. In many cases, inadequate mixing cannot be overcome simply by increasing the mixing time. Mixer selection, rotation speed, capacity and fill level are all subject to optimization, as they can all affect the blend homogeneity (Alexander et al., 2004). For example, the incorporation of a large proportion of fine lactose (20%) improved considerably the aerosol performance but reduced greatly the content uniformity of fluticasone propionate when the blending was performed in the Turbula mixer (Relative standard deviation, RSD > 20% after 20 min independent of the selected speed). Achievement of a homogeneous mixture (RSD < 3%) required the use of powerful shear mixers (Colette MP-20 or Mi-Pro) operating at moderate rotational speeds to avoid the phenomenon of powder segregation and dust generation (Sebti et al., 2007).

Different powders may have different mixing requirements depending on the interactive forces present among the various particles. For low concentration (drug–carrier ratio) blends, geometric dilutions are necessary, using multiple pre-blending steps. Various blending options are available: low-energy tumbling blending; tumbling blending with sieving to break up agglomerates of micronized drug and aid distribution of the powder mass; and high-energy blending, with paddles, impeller blades or planetary augers actively cutting the powder bed and redistributing powder within the blending vessel (Malcolmson and Embleton, 1998).

An interactive mixture of the two components is prepared by blending until the mixture can remain intact during the filling process (to produce an accurate metered dose) and then freely separate into its primary components during inhalation. There, the drug particles separate from the carrier particles and are carried deep into the lungs while the larger carrier particles impact the

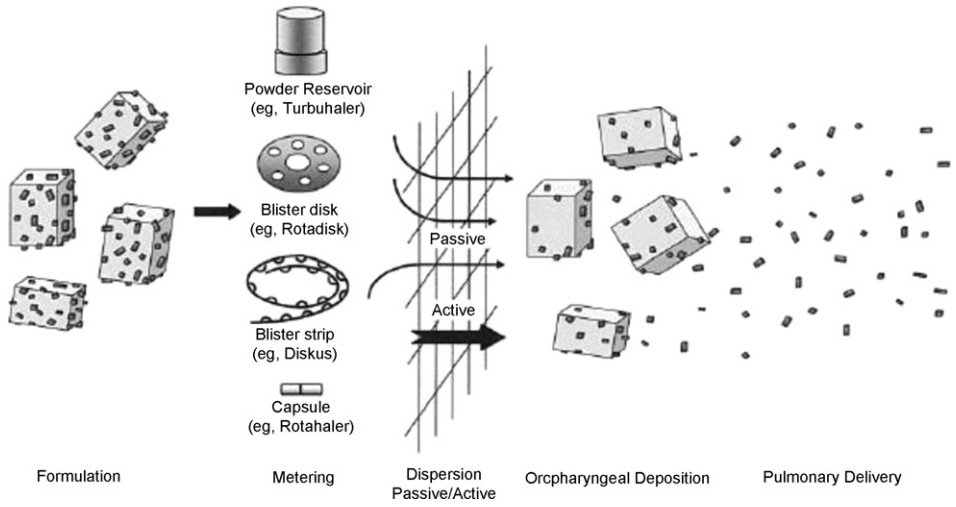

Fig. 2. Principle of dry powder inhaler design: Blending of active substance and coarse carrier, deaggregation within inspiratory flow and pulmonary deposition of the drug (Telko and Hickey, 2005).

oropharynx and are cleared. Inadequate drug/carrier separation is one of the main explanations for the low deposition efficiency encountered with DPIs (Zeng et al., 2000). The homogeneity of the blend and the deaggregation and dispersion properties of the respirable particles upon activation (driven by the patient's inspirational energy) are, on a microscopic scale, governed by the resulting cohesive (drug–drug) and adhesive (drug–excipient) interaction forces within the formulation.

In fact, the adhesion force must be sufficient to avoid demixing during metering but small enough to allow detachment during aerosolization. Excessive adhesive forces may prevent elutriation of the respirable particles from the carrier surfaces, leading to upper airway deposition. Similarly, strong cohesive forces may enhance agglomerate formation, which could directly affect the fluidization and dispersion characteristics of the formulation (Begat et al., 2004a). So, attention must be paid to pre-blending steps and optimization of the mixing parameters. Furthermore, particle engineering with modification of the surface of the particles may be a useful tool in order to control the forces of interaction. 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 yet allows easy separation during inhalation.

5.3.2. Redispersion

Availability of the active drug depends on the redispersion of the particles in the inspired air, which is a function of the cohesive forces between drug particles and the adhesive forces between drug and carrier particles. Larger carrier particles deposit on the oropharynx while fine drug particles partly reach the deep lung. Deaggregation principally occurs in two regions: the device and the oropharynx. The majority of DPIs are therefore composed of short tubes and complex geometries, with often the presence of a grid, through which an airflow passes (Fig. 2). In function of the device conception (presence of a grid or tortuous channels, rotation of the capsule, rumbling or spinning motions, etc.) different forces (collision, turbulence, shear, friction, inertial, impaction forces, etc.) are used for the redispersion of the powders. Interestingly, studies using computational fluid dynamics have been made on DPI devices in order to evaluate the influence of the mouthpiece geometry, the mouthpiece length, the grid structure, the air inlet size and the role of the capsule (Coates et al., 2004). These studies have shown that when modifications to the design of the device are made, the flow-field generated within the device can change significantly, and that these changes may have a significant effect on the overall performance of the inhaler. It is known that there is a correlation between the turbulence velocity and the deaggregation that occurs: as the turbulence increases, the deaggregating force increases. In fact, the deagglomeration of drug particles to form a fine respirable aerosol cloud is thought to be achieved by three major mechanisms: Particle interaction with shear flow and turbulence, particle–device impaction, and particle–particle impaction. These adhesive forces must be carefully considered since inadequate separation of drug and carrier is the main reason for deposition problems.

In general, the morphology and roughness of carrier particles are not uniform; that is, they contain regions that exhibit different roughness parameters. Clearly, these variations in physicochemical properties at the surface of a carrier material may lead to differences in the apparent adhesion properties of drug particles. Furthermore, during the dynamic process of mixing, adherence of drug particles to more adhesive areas of the carrier surface is likely to occur. Indeed, Hersey (1975) proposed that the surfaces of larger particles consisted of distinct regions containing so-called "active sites". It was further suggested that when the amount of fine carrier particles in the mixture is below the saturation limit of the large carrier particles' adhesive potential, the fine particles will preferentially bind to these active sites. When these active sites have been completely occupied by fine particles, a binary carrier system will then exist: carrier with strongly bound fine particles and carrier with weakly bound fine particles or free fine particles.

This presence of active sites has obvious implications for DPI drug delivery since retention of drug particles on these relatively high-energy sites during processing and aerosolization would result in a decrease in the apparent respirable drug fraction, as suggested by Staniforth (1996). Furthermore, it is suggested that the active sites present on the surface of the carrier will have a specific energy distribution with a critical average adhesion point below which particles – drug or lactose – could be removed.

Current methods for overcoming such issues include "filling" the potential active sites by increasing the fine particle content present on the carrier surface (Zeng et al., 1999) or pacifying the effects of active sites by the addition of so-called "force control agents". This additional or ternary component can be added to occupy and pre-saturate higher-energy binding sites on the carrier before the drug is added consequently increasing the release and respirable fraction of the active drug. In fact, various techniques have been applied to smooth and/or to modify carrier particle surfaces, e.g., dry coating with hydrophobic lubricants such as sucrose

tristearate (Iida et al., 2003a) and magnesium stearate (Young et al., 2002; Ferrari et al., 2004); lipid coating with hydrophobic properties (Pilcer et al., 2006); wet coating with hydrophilic polymers such as hydroxypropylmethylcellulose (Iida et al., 2005); surface dissolution with organic solvents like 70% ethanol (Iida et al., 2003b); or a combination of these techniques (Young et al., 2002). While the above approaches appear to hold promise, the safety of hydrophobic excipients, such as sucrose tristearate, for use in the lungs has yet to be established; their clearance mechanisms from the lungs are not well understood. Nevertheless, magnesium stearate is used in the approved Skyeprotect technology to protect the drug from moisture for inhalation use.

In summary, as excipients can make up over 99% of a product's weight, their selection is a crucial determinant of overall DPI performance. Despite the apparent lack of choices, the excipient must be carefully selected given that its physicochemical properties, such as size and morphology, profoundly affect the performance of the formulation.

5.3.3. Nature of excipients

The primary function of the lungs is respiration. To fulfill this purpose, the lungs have a large surface area and thin membranes. Many compounds that could enhance drug delivery outcomes also have the potential to injure the lungs (Telko and Hickey, 2005). Polymers such as methylcellulose or cross-povidon or anionic surfactants that are usually used in oral formulations, cannot be delivered to the lungs because of their non-degradability or irritating nature. It should be noted that the current excipients approved by the Food and Drug Administration for respiratory drug delivery are very limited in number and not accepted worldwide. Excipients accepted in nebulization and MDI formulations (Tables 1 and 2) such as polysorbates, sorbitan trioleate or oleic acid are not widespread in DPI development due to their low melting point and their semi-solid or liquid state. Table 3 presents a summary of excipients already approved for pulmonary delivery or presented as interesting alternatives for DPI formulations. The array of potential excipients is limited to compounds that are biocompatible or endogenous to the lung and can easily be metabolized or cleared. If particles deposit throughout the conducting airways, they are removed by mucociliary clearance. Particles will be eliminated in 24–48 h according to the number of cilia and their beat frequency. In the bronchioles and alveoli, particles will be cleared by direct absorption through the epithelial cells or uptake by the alveolar macrophages (Dalby et al., 1996).

5.3.3.1. Lactose and other sugars. Currently, lactose is the most commonly used excipient in marketed DPIs (Beclophar®, Flixotide®, Relenza®, Seretide®, Spiriva®, Symbicort®). It has an established safety and stability profile, different manufacturing processes with tight controls over purity and physical properties, is easily available at different grades and is inexpensive (Smyth and Hickey, 2005). Published inhalation studies in humans have shown no local effects of lactose by inhalation. As the particles of lactose in these preparations are generally larger than the respirable range, the bulk of lactose will be deposited in the mouth and throat where it is hydrolyzed by enzymes of bacterial origin present in normal saliva or swallowed to be subsequently metabolized by intestinal enzymes. Concerning the fine particles of lactose reaching the lungs, they are rapidly absorbed and metabolized by the intestinal epithelium and are principally excreted in urine (Clark et al., 1974). Furthermore, in contrast to oral administration, lactose swallowed at the levels present in inhaled preparations (up to 25 mg) is unlikely to present problems even in patients with lactose intolerance (Baldrick and Bamford, 1997).

Nearly all DPI products already on the market rely on lactose as a carrier material. As can be seen in Table 4, manufacturers utilize different physical forms of lactose for inhalation such as α-lactose monohydrate or anhydrous β-lactose, obtained by various techniques such as milling or spray drying and presenting a wide range of particle-size distribution (Vanderbist et al., 1999).

In order to investigate the possibility of using inhalation grade anhydrous lactose in carrier-based formulations, a 200 μg dose blend of budesonide was prepared using four lactose grades (Fig. 3): ML001 (DMV-Fonterra Excipients, The Netherlands), LH200 (Friesland Foods Domo, The Netherlands), Monohydrate 120M and Anhydrous 120 MS (Sheffield Pharma Ingredients, USA). The fine particle dose (FPD) of budesonide was about 49, 35 and 25 for ML001, LH200 and both Monohydrate 120M and Anhydrous 120MS, respectively. The aerosolisation of budesonide from a formulation containing inhalation grade anhydrous lactose was

Table 4
List of commercially available inhalation grade lactose.

	Description	$D(0.5)$ (μm)	$D(0.9)$ (μm)
Respitose ML001 (DMV-Fonterra)	Milled lactose monohydrate with broad PSD	55	170
Respitose ML006 (DMV-Fonterra)	Fine milled lactose monohydrate with narrow PSD	17	45
Respitose SV003 (DMV-Fonterra)	Sieved lactose monohydrate with narrow PSD	60	100
Respitose SV010 (DMV-Fonterra)	Coarse sieved lactose monohydrate with broad PSD	105	175
Lactohale 100 (Domo)	Sieved lactose monohydrate with narrow PSD and tomahawk shaped particles with smooth surface	125–145	200–250
Lactohale 200 (Domo)	Gently milled lactose monohydrate with irregular shaped particles	50–100	120–160
Lactohale 201 (Domo)	Hardly milled lactose monohydrate with irregular shaped particles	50–100	120–160
Lactohale 300 (Domo)	Micronized lactose monohydrate with narrow PSD	<5	<10
Inhalac 70 (Meggle)	Sieved crystalline lactose with narrow PSD	200	300
Inhalac 120 (Meggle)	Sieved crystalline lactose with narrow PSD	150	200
Inhalac 230 (Meggle)	Sieved crystalline lactose with narrow PSD	100	140
Anhydrous 120 MS (Sheffield Pharma Ingredients)	Sieved anhydrous lactose	–	–
Monohydrate 120 MS (Sheffield Pharma Ingredients)	Sieved monohydrate lactose	–	–
Anhydrous 40M (Sheffield Pharma Ingredients)	Milled anhydrous lactose	–	–
Monohydrate 40M (Sheffield Pharma Ingredients)	Milled monohydrate lactose	–	–
Monohydrate 80M (Sheffield Pharma Ingredients)	Milled monohydrate lactose	–	–
Monohydrate 120M (Sheffield Pharma Ingredients)	Milled monohydrate lactose	–	–

$D(0.5)$ is the size of particle below which 50% of the sample lies (volume median diameter) and $D(0.9)$ is the size of particle below which 90% of the sample lies.

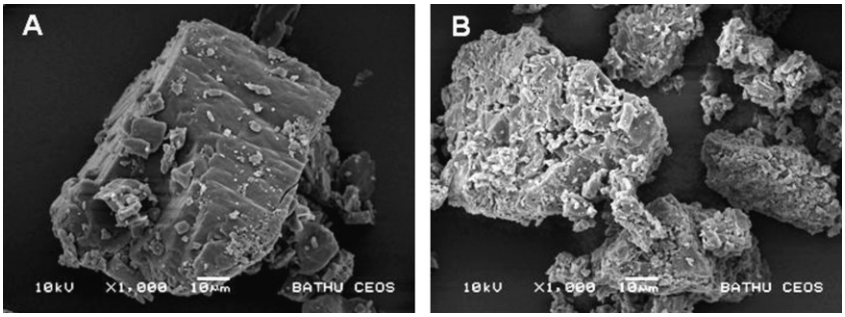

Fig. 3. SEM of inhalation grades of (A): lactose monohydrate (LH200) and (B) anhydrous lactose (Anhydrous 120 MS) (Pitchayajittipong et al., 2010).

significantly less than formulations employing inhalation grades of lactose monohydrate, ML001 and LH200. Even though there was no apparent relationship between characteristics such as powder density or particle size and aerosolisation, there did appear to be a relationship between carrier rheological properties and aerosolisation (Pitchayajittipong et al., 2010). It has been shown that the crystallinity of lactose plays an important role; having high-energy surfaces, amorphous lactose exhibits strong adhesive interactions with drug particles, leading to low inhalation efficiency. Therefore, α-lactose monohydrate is the crystalline form most commonly employed as a drug carrier in DPIs. But lactose may not be the carrier of choice due to its sugar-associated reducing function that may interact with functional groups of drugs such as formoterol or peptides and proteins. It is therefore reasonable to look for alternative carriers that still possess the beneficial properties but overcome the aforementioned drawbacks of lactose.

Other sugars have been tested as potential carriers in DPIs, such as mannitol, glucose monohydrate, trehalose, dextrose, maltose, sorbitol, maltitol and xylitol (Tee et al., 2000; Mao and Blair, 2004; Steckel and Bolzen, 2004; Glover et al., 2008). Anhydrous glucose is already used in the marketed product Bronchodual® (Boehringer) and mannitol was used in a drug formulation for DPI insulin delivery (Exubera®, Pfizer) which was until recently available on the market. Nevertheless, a major problem with these sugars, especially with the more hygroscopic substances – sorbitol, maltitol and xylitol – has seemed to be their sensitivity to humidity. Indeed, several studies have suggested that non-surface-specific capillary forces play a dominant role in adhesion between drug and carrier, which may significantly reduce the deaggregation and dispersion properties of a dry formulation (Price et al., 2002; Young et al., 2003). A study has shown that after exposure to 75% RH at 25 °C for up to 6 days, dry powder formulations containing a micronized salbutamol base and a fraction of sorbitol, dextrose or spray-dried maltose as a carrier were found to take up a large quantity of water (15–40%), which resulted in a marked change in the surface texture of the particles and a drastic reduction in the FPF. In contrast, the formulation containing lactose monohydrate was found to only take up moisture on its surface, and there was only a small reduction in its FPF after storage at 75% RH (Young et al., 2007; Zeng et al., 2007). In fact, the capillary forces, arisen from the dynamic condensation of water molecules onto particle surfaces, seem to be less pronounced with mannitol and lactose as carriers, which might be due to the fact that these sugars are less hygroscopic than the other carbohydrates. Different forms of mannitol, lactose and maltitol were mixed with either terbutaline sulfate or formoterol fumarate, and crystallized mannitol produced the highest fine particle dose of terbutaline sulfate (Saint Lorant et al., 2007).

Therefore, mannitol, which is a sugar alcohol previously inhaled for diagnosis of bronchial hyperresponsiveness, has emerged as a promising carrier. In addition, mannitol was shown to stimulate the reserve capacity of the mucociliary system (Daviskas et al., 2008) and to enhance clearance of mucus (Jaques et al., 2008). Studies on the effect of mannitol particle size on lung deposition have already been done (Glover et al., 2008).

Different studies on the influence of the presence of mannitol and lactose during spray drying have been published with contradictory results. On the one hand, Maa et al. (1997) demonstrated that the addition of lactose and mannitol showed FPFs about 30% whereas the addition of trehalose produced powder with poor aerosolization properties. The authors conclude that the addition of different excipients allows the production of particles with a suitable morphology. On the other hand, studies showed that powders with lactose yield the highest deposition values whereas powders with mannitol exhibit lower FPFs due to the cohesive nature of the excipient (Bosquillon et al., 2001). In fact, mannitol has been shown to increase the FPFs of spray-dried powders, although a large amount of mannitol results in a decrease in aerosolization performance. In contrast to Maa et al. (1997), Bosquillon et al. (2001) demonstrated that the nature of the excipient added did not affect the size, density or morphology of the spray-dried particles, but it influenced the aerosolization properties. It is important to note that the study evaluating different carriers was conducted with one drug only. Each drug/carrier pair and their interaction with the inhalation device must be taken into account; it is not reasonable to assume identical behavior of the dry powder formulation for different drugs based on the performance of one.

Aside from accepted sugars such as lactose, glucose and mannitol, sugar such as trehalose has been shown to be feasible alternatives to lactose, and it is expected that it will eventually find his way into approved products. An additional benefit that may be gained from the use of a sugar carrier is the taste/sensation on inhaling, which can assure the patient that a dose has been taken.

Interestingly, the flow properties of sugars in general could be improved by adding hydrophobic silicon dioxide. The addition of 2% (w/w) Aerosil R972 had the effect of increasing the FPF from 8% to 42% when sorbitol is used as carrier. The effect of Aerosil in the powder blend is twofold: firstly, the surface of the carrier is covered by the nano-sized silica particles, which reduces the contact area of drug and carrier; secondly, the drug particles themselves are covered with the nano-sized Aerosil particles, which results in reduced cohesion forces hence better dispersibility (Steckel and Bolzen, 2004). Nevertheless, Aerosil, which is amorphous silica, may not be suitable as a carrier for inhalation since its toxicology profile reveals potent carcinogenic activity leading to strong carcinogenesis in the lungs.

Nevertheless, excipients that modify hygroscopic properties of drug particles, such as cholesterol or lipids, may need to be considered (Hickey et al., 1990).

5.3.3.2. Lipids. In the inhalation field, excipients considered as GRAS (generally recognized as safe) can comprise materials that are endogenous to the lungs and locally present in large quantities

(in order to avoid the normal concentrations being altered by the external input) such as phosphatidylcholine. In fact, the surfactant present in the lungs is composed by weight of approximately 90% lipids and 10% proteins. The lipids are characterized by an unusually high level of saturated fatty acid chains such as the predominant dipalmitoylphosphatidylcholine (DPPC), which represents 40% by weight of lung surfactant and contributes substantially to the unique properties of pulmonary surfactant. In addition to DPPC, lung surfactant contains unsaturated phosphatidylcholines (~35%), phosphatidylglycerol (~10%), phosphatidylinositol (~2%), phosphatidylethanolamine (~3%) and sphingomyelin (~2.5%). There is also a small amount (~3%) of neutral lipid composed of mainly cholesterol (Possmayer et al., 2001).

Solid lipid microparticles (SLM) composed of compritol were tested by intratracheal administration in rats as a potential application as carriers in pulmonary drug delivery (Sanna et al., 2004). Since results did not show a significant inflammatory airway response, compritol microparticles might be a potential carrier for encapsulated drug via the pulmonary route.

Other hydrophobic excipients such as glyceryl behenate and tripalmitin were used to form sustained release microspheres of terbutaline sulfate by spray drying and exhibited an FPF of approximately 46%. Unfortunately, release of the microspheres was not significantly different in simulated lung fluids than for raw terbutaline sulfate (Cook et al., 2005).

Liposomes are one of the most extensively investigated systems for controlled delivery of drug to the lung given that they can be prepared with phospholipids endogenous to the lung. Indeed, lipids such as egg phosphatidylcholine (PC), distearoyl phosphatidylcholine (DSPC) and DPPC may be used to form liposomes, which are small vesicles with an aqueous compartment enclosed by one or more concentric lipid bilayers.

A significant disadvantage of many existing inhaled drugs is the relatively short duration of resultant clinical effects, which requires most medications to be inhaled at least twice daily. This often leads to poor patient compliance. A reduction in the frequency of dosing would be convenient, particularly for chronic diseases such as asthma. The advantages of such an approach include reduced dosing, increased effectiveness of rapidly cleared medicine and enhanced residence time at the target site for the treatment of infection. Many challenges exist in developing controlled release inhalation medicine, which is reflected in the fact that no commercial product exists. Liposomes can be produced with a wide range of sizes and are able to encapsulate a variety of both hydrophilic and lipophilic drug molecules in their core or within their membrane bilayers. Cytotoxic agents, bronchodilators, anti-asthma drugs, antimicrobial and antiviral agents and drugs for systemic action, such as insulin and proteins, have been investigated (Zeng et al., 1995; Desai et al., 2003; Changsan et al., 2009). Liposomal formulations of amikacin were administered to three healthy volunteers by nebulization. Total lung deposition, expressed as a percentage of the emitted dose, was $32.3 \pm 3.4\%$. The overall mean retention at 24 and 48 h was 60.4 and 38.3% of the initial dose deposited, respectively (Weers et al., 2009).

Liposomes are known to promote an increase in drug retention time and reduce the toxicity of drugs after administration. Several factors have been shown to influence drug release and absorption of liposome encapsulated drugs within the lung, such as the composition of lipids and the size of the liposomes used. For example, the presence of cholesterol and phospholipids with saturated hydrocarbon chains increased the drug residence time within the lung for terbutaline (Abra et al., 1990). The presence of both of these components is known to decrease liposome membrane permeability to encapsulated drug and to protect liposomes from *in vivo* destabilization. The effect of phospholipids on pulmonary insulin delivery and absorption by liposomes was examined in rats. Although liposomes with phospholipids devoid of the palmitoyl group (DSPC, dilauroyl phosphatidylcholine (DLPC), dimyristoyl phosphatidylcholine (DMPC) or dioleoyl phosphatidylcholine (DOPC) had no delivery-enhancing effects, liposomes with DPPC (palmitoyl group content: 100%) or egg yolk phosphatidylcholine (EPC) (palmitoyl group content: 32.5%) enhanced insulin delivery, and enhancement was dependent on the palmitoyl group content of the phospholipids. These findings suggest that liposomes with DPPC enhance pulmonary insulin delivery by opening the epithelial cell space in the pulmonary mucosa and not via mucosal cell damage (Chono et al., 2009). Liposomes with DPPC could be useful as a pulmonary delivery system for peptide and protein drugs.

Considering the influence of the size of the liposomes, it is important to note that inhaled liposomes with different particle sizes will deposit at different sites in the respiratory tract, leading to complications in determining the factors that affect the pharmacokinetics of drugs within the lung. They are commonly delivered either in aqueous form via nebulization (Finlay and Wong, 1998; Zaru et al., 2007) or in dry powder form (Desai et al., 2003; Changsan et al., 2009). Dry powder liposome formulations are generally prepared by lyophilization of the aqueous liposome dispersions followed by micronization by jet milling to achieve particles in the desired range. However, this process could cause deleterious effects on liposome integrity, thereby causing leakage of the entrapped drug (Desai et al., 2003).

Formulations with various phospholipids – such as DPPC, DMPC, DSPC and dipalmitoyl phosphatidyl glycerol (DPPG) – and carbohydrates have already been tested as liposome carriers for superoxide dismutase (Lo et al., 2004). The formulations with such lipids demonstrated superior aerosolization characteristics compared to formulations prepared using sugars, although the combination of carbohydrate and phospholipids proved the most successful with a formulation prepared using sucrose and DPPC providing the highest FPF: 72% of the emitted dose.

Human A549 alveolar cell viability after 24 h exposure was not affected by DPPC and DSPC (14 and 13 mg/ml, respectively) empty liposomes; however, a slight reduction in cell viability was caused by PC empty liposomes (Zaru et al., 2007). By comparing different liposome types, it is interesting to note that phosphatidylglycerol (PG)-containing liposomes are always significantly more toxic compared to those containing the same phospholipids devoid of PG (Zaru et al., 2009).

A study has shown that the excipient type and its relative proportion greatly affects the *in vitro* deposition of dry powders. For example, a powder consisting of an albumin/lactose/DPPC mixture (30/10/60, w/w/w) showed particularly efficient aerosolization performance (Bosquillon et al., 2001). Each excipient in the formulation appeared necessary for the achievement of optimal aerosolization properties. DPPC might facilitate droplet formation in the atomization step of spray drying as well as likely decrease particle surface energy thereby decreasing powder cohesiveness (Vanbever et al., 1999). The presence of DPPC also seemed to influence the morphology of the particles; powders containing up to 30% (w/w) DPPC exhibited a torroidal particle shape whereas powders with 60% (w/w) DPPC were sponge-like and powders with 90% (w/w) were spherical (Vanbever et al., 1999). Lactose and albumin might provide the skeletal structure necessary for the formation of solid particles by spray drying, which could not be provided by DPPC alone. Albumin possesses surfactant properties and may limit point to point contacts due to highly indented geometries, reducing interparticle adhesion forces (Bosquillon et al., 2001). Nevertheless, albumin, being a protein, possesses limited shelf stability and can induce the production of auto-antibodies following repeated administration. Moreover, human sources of albumin carry risks of toxicity and infection due to impurities (Minne et al., 2008).

Phospholipids and cholesterol have also been used in formulations as solid lipidic carriers or fillers (Sebti and Amighi, 2006). These new formulations were developed using solid lipid microparticles (SLmP) that are composed of DSPC and cholesterol, two physiologically well-tolerated components, as a pharmaceutically acceptable filler and/or carrier to improve drug targeting to the lung. Indeed, SLmP may reduce local irritation, offering acceptable tolerance in the pulmonary tract as they are mainly constituted of biocompatible and biodegradable material (Myers et al., 1993). Moreover, the hydrophobic nature of neutral lipids (cholesterol) reduces the absorption of the ubiquitous vapor leading to a reduction in the aggregation and the adhesion of particles. The SLmP were obtained by spray drying and were formulated as lipidic matrices entrapping budesonide or as physical blends (drug carrier). The phase transition temperature (T_c) of the phospholipids plays a crucial role in the size characteristics of phospholipids-based powders produced by spray drying. As the phase transition temperature of the phospholipids increases, the MMAD of particles decreases. In this respect, Phospholipon 90H® is preferred to other commercially available phospholipids, as it exhibits one of the highest T_c values (around 54 °C) (Sebti and Amighi, 2006). In a comparative clinical study in six healthy volunteers, it was estimated that lung deposition was 1.5 and 2.0 times higher for the lipidic matricial and the lipidic physical blend formulations containing a 90:10 cholesterol:DSPC ratio, respectively, than for the Pulmicort Turbuhaler (Sebti et al., 2006).

In another study, the same composition of lipids was used for coating tobramycin particles to improve drug targeting to the lung. For pulmonary delivery, the coating of micron-sized drug particles are very interesting, allowing a high drug loading while minimizing possible accumulation of water-insoluble excipients in the lungs. Moreover, the drug is protected by a layer of coating material that protects against possible degradation or for control released delivery. Lipids coat the drug particles with hydrophobic films that protect a hygroscopic drug like tobramycin from humidity. Lipid deposition results in a modification of the surface properties of micron-sized tobramycin particles, which enables deep deposition in the lungs. The evaluation of the influence of the coating level showed that a deposition of only 5% (w/w) lipids (on a dry basis) is sufficient to improve particle dispersion properties during inhalation. These results reveal the need to add sufficient amounts of covering material to significantly modify particle surface properties and reduce their tendency to agglomerate while limiting the lipid level in the formulations to avoid any undesirable sticking and to allow the delivery of more of the active drug to the deep lung. The FPF, which was approximately 36% for uncoated micronized tobramycin, increased to up to approximately 68% for the most effective lipid-coated formulation (Pilcer et al., 2006). In a comparative clinical trial in nine cystic fibrosis patients, the percentage of dose (mean ± SD) in the whole lung was 53.0 ± 10.0% for the lipid-coated formulation, 34.1 ± 12.4% for the non-coated formulation and 7.6 ± 2.7% for the comparator product, Tobi® (Pilcer et al., 2008). In addition, the results indicated that the inhalation of 25 mg of tobramycin alone or in the presence of lipids did not have any significant effects on lung function. No major or minor complications were observed during the clinical trial and there was no evidence of bronchoconstriction, suggesting good tolerance of these products. A major advantage of these two products is that they are biodegradable and biocompatible within the lungs.

In fact, lipids can be used to modify the initial properties of drug particles to enhance the desagglomeration properties of a powder and deposition deep in the lung or to achieve controlled release pulmonary therapy. Moreover, lipids in general are the excipients of choice because they are mostly endogenous to the lung and can easily be metabolized or cleared.

5.3.3.3. Amino acids. Besides sugars and lipids, several alternative excipients have been tested, such as amino acids: glycine, alanine, leucine and isoleucine. Recently, amino acids have been shown to decrease hygroscopicity and improve surface activity and charge density of particles. The addition of various amino acids to formulations for inhalation obtained by spray drying has been demonstrated to significantly improve the *in vitro* deposition profile of a dry powder (Li et al., 2005; Seville et al., 2007). Moreover, amino acids can also protect proteins against thermal stress and denaturation (Arakawa et al., 2007). Histidine, lysine, glycine and arginine have been used as a cryoprotectant for various peptides and proteins such as factor VIII, human growth hormone (HGH), human anti-interleukin 8 monoclonal antibody and interleukin 2 (IL-2), increasing the stability in lyophilization and decreasing aggregation upon reconstitution (Chen et al., 2003). Amino acids such as histidine and glycine have already been used as buffers for proteins such as human growth hormone and antibodies. Moreover, all powders that contain various amino acids, such as arginine, aspartic acid, phenylalanine and threonine, exhibit better aerosolization characteristics compared to raw powder (Li et al., 2005). Different studies that include various amino acids have demonstrated that the addition of leucine yields the best results in term of aerosolization (Rabbani and Seville, 2004; Chew et al., 2005; Seville et al., 2007). For example, selection of appropriate solvent systems and leucine concentration allows the preparation of spray-dried powders that display enhanced aerosolization properties of β-oestradiol powders (Rabbani and Seville, 2005). The influence of the amount of leucine in the powder has been demonstrated in various papers. The consensus is that 10–20% (w/w) of leucine in spray-dried solutions of ethanol or water give optimal aerosolization characteristics of powders containing peptides or sodium cromoglycate (Chew et al., 2005; Rabbani and Seville, 2005; Padhi et al., 2006). It seems that addition of leucine results in less cohesive particles and a possible decrease in particle size due to the surfactant behavior of leucine, reducing the size of droplets produced during atomization. Spray-dried isoleucine has also been shown to improve the aerosol performance and stability of various formulations.

Recently, trileucine has also been proven to be an efficient surface active agent. As a result of its surface activity, trileucine molecules can orient the hydrophobic groups towards the air at the air/liquid interface during the drying process, providing a hydrophobic surface to the dry particle, thereby contributing to the observed improved aerosol efficiency. Addition of trileucine to spray-dried particles for inhalation produced corrugated particles of low cohesivity (Lechuga-Ballesteros et al., 2008).

Using leucine, a novel method for improving drug absorption is the formulation of effervescent spray-dried powders using sodium carbonate, citric acid, lactose as a bulking agent and PEG 6000 as a lubricant. This system was found to give greater release of ciprofloxacin compared to a simple lactose spray-dried powder (56% vs. 32%, respectively) (Ely et al., 2007).

Unfortunately, despite very active research on formulations and the potential of amino acids in pulmonary drug delivery, little is known about the local toxicity of various amino acids and their systemic absorption after pulmonary administration. Nevertheless, as amino acids are endogenous substances, they might not present major risk of toxicity to the lungs.

5.3.3.4. Surfactants. Surfactants such as polyoxyethylene sorbitan fatty acid esters (Polysorbates) and sorbitan esters have been widely used in formulations for nebulization and metered-dose inhalers. However, their use in dry powder formulations is not widespread due to their low melting point and their semi-solid or liquid state. Nevertheless, a new approach to preparing low density particles involves poloxamer or phosphatidylcholine as

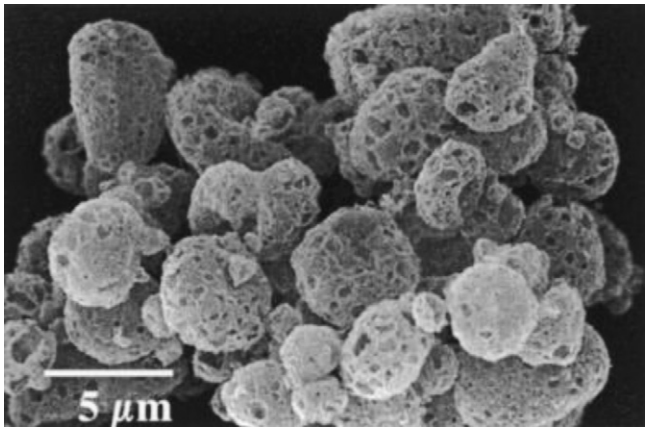

Fig. 4. SEM of PulmoSpheres® of budesonide (Duddu et al., 2002).

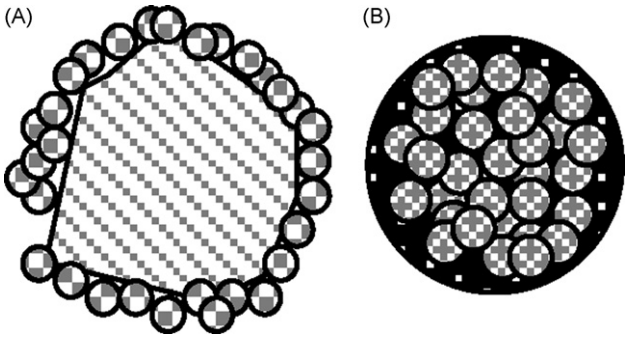

Fig. 5. Formulations composed of tobramycin nanoparticles: (A) micron-sized particles coated with nanoparticles and (B) agglomerates of nanoparticles (Pilcer et al., 2009b).

suitable surfactants. It has been demonstrated that the presence of poloxamer in the feed emulsion results in particles with improved dispersibility and a higher fine particle fraction (Steckel and Brandes, 2004). Poloxamer was also used to produce large, light respirable powders prepared by spray drying, which contain leucine and tobramycin sulfate (Padhi et al., 2006) or thymopenton (Wang et al., 2009). It is interesting to note that 2% of poloxamer 188 significantly improved powder flowability. Moreover, a dry powder of itraconazole, polysorbate 80 and poloxamer 407 in a 1:0.75:0.75 ratio was administered to mice by nebulization every 12 h for up to 12 days. The results have shown that aerosolized administration of amorphous itraconazole and excipients does not cause inflammation or changes in pulmonary histology and is not associated with pro-inflammatory cytokine production (Vaughn et al., 2007).

Micron-sized hollow porous microspheres (PulmoSpheres®) are prepared using a two-step process. First, an emulsion is prepared by high-pressure homogenization using saturated phosphatidylcholine as the surfactant. The emulsion is then combined with a second aqueous solution containing the active agent and another wall-forming material (e.g., co-surfactants such as PVA or HPMC, sugars and salts). The second step involves spray drying the aqueous dispersion. The oil phase functions as a "blowing" or "inflating agent" during spray drying, which retards droplet shrinkage during the drying process and simultaneously creates pores and voids in the particles. Mean geometric and aerodynamic diameters are about 5 and 7 μm, respectively, and the bulk density is about 0.4 g/cm^3. Morphologically, the particle walls have a sponge-like appearance with pores (Fig. 4) (Duddu et al., 2002). Particle size, morphology and density can be controlled through the selection of the blowing agent type and its concentration. High respirable fractions of active substances such as tobramycin sulfate and budesonide are obtained *in vitro* as a result of a significant decrease in interparticle attractive forces and improved aerodynamic properties due to the hollow porous design. One phase I clinical evaluation of dry powder tobramycin on healthy volunteers using lipid-based Pulmosphere technology has already been conducted; a mean whole lung deposition of 34 ± 6% was reported (Newhouse et al., 2003).

Using high-pressure homogenization and spray drying techniques, novel formulations have been developed for manufacturing dry powders for inhalation composed of a mixture of micro- and nanoparticles to enhance lung deposition and produce high therapeutic payload particles (Pilcer et al., 2009b). DSPC, sodium taurocholate and sodium glycocholate have been tested as an appropriate surfactant system in isopropanol for the stabilization of tobramycin nanoparticles. The nanoparticles produced were used to coat micron-sized particles of the drug to modify its surface properties and to decrease the agglomeration tendency of the powder. On the other hand, formulations composed solely of nanoparticles have been produced to form easily dispersible and reproducible micron-size agglomerates of particles with low density and high porosity during inhalation (Fig. 5). To retrieve nanoparticles from a dried-powder state, nanosuspensions with different concentrations of Na-glycocholate were spray dried. The fine particle fraction increased from 36% for the raw micronized tobramycin material to about 61% for the most effective formulation (Pilcer et al., 2009b). One of the major advantages of these formulations is the low level of excipient used (less than 5% expressed as a percentage of the active drug), allowing for a high drug concentration at the site of action and minimizing the potential toxicity of the excipients in the lung.

5.3.3.5. Absorption enhancers. Many potent novel absorption enhancers have been investigated for the systemic administration of various peptides and proteins to the lungs (Hussain et al., 2004). However, the mechanism of action of these promoters could be due to an irreversible distortion of the alveolar epithelial cell layer, which could potentially make the lungs susceptible to the entry of exogenous allergens and dust particles inhaled during respiration. Thus, the use of absorption enhancers in pulmonary drug delivery has generated safety concerns regarding possible long-term effects.

Cyclodextrines (CDs) have been shown to provide many benefits in pulmonary drug delivery, such as improvements in aqueous solubility, systemic absorption and bioavailability of drugs. CDs introduce hydrophobic drug molecules or hydrophobic parts of drugs into their apolar cavities, thereby conferring the potential to modify the properties of the inhaled drug. CDs have been described in controlled release systems or as adjuvants in the design of new formulations such as liposomes, microspheres and nanoparticles (Challa et al., 2005). CDs have been reported as potential absorption enhancers in pulmonary delivery of proteins, such as human growth hormone, insulin, cyclosporine A, etc. (Hussain et al., 2004; Ungaro et al., 2006; Jalalipour et al., 2008). The effect of CDs on respiratory cell metabolic activity and permeability *in vitro* was shown to be concentration-dependent and variable according to the type of CD, the type of chemical derivatization and the degree of substitution (Salem et al., 2009). It has been shown that CDs in general do not cause a decrease in cell viability at concentrations less than or equal to 1 mM whereas differences between various CDs were observed at concentrations greater than or equal to 2 mM. In terms of local cytotoxicity, hydroxypropylated-β-CD and natural γ-CD seemed to be the safest while randomly methylated-β-CD was the least safe for pulmonary delivery. CDs may also be absorbed into the systemic circulation after inhalation, but more studies are needed to clarify this point. In rabbits, systemic bioavailabilities of 66–80% have been reported with intratracheally

administered β-CDs, dimethyl-β-CDs and hydroxypropyl-β-CDs (Matilainen et al., 2008). Nevertheless, little has been published concerning the potential toxicity of cyclodextrin formulations on the lung parenchyma and bronchial tree.

Various protease inhibitors have been investigated, among which are nafamostat mesilate or bacitracin. Studies have shown that when nafamostat was administered with insulin, the relative bioavailability of the peptide was approximately twice that obtained without a promoter. Nevertheless, the administration of nafamostat compared to other absorption enhancers, such as bile salts, produced modest effects (Okumura et al., 1992).

Among the surface active agents, bile salts, such as sodium salts of cholate, deoxycholate, glycocholate, glycodeoxycholate, taurocholate and taurodeoxycholate, are commonly employed as absorption enhancers (Hussain et al., 2004). They increase transcellular transport via interaction with or fluidization of the cell membrane, which subsequently makes it more permeable. For example, the administration of sodium taurocholate with insulin resulted in an increase in bioavailability from $2.6 \pm 0.3\%$ to $23.2 \pm 4.4\%$. The main mechanisms behind the absorption enhancement are the production of insulin monomers and an opening of tight junctions between adjacent airway epithelial cells (Johannson et al., 2002). Although the results are very promising, the use of high amounts of bile salts may not be feasible for chronic use since they may damage the epithelial surface. Specifically, sodium taurocholate showed a concentration-dependent toxicity when administered to Caco-2 cell monolayers. Concentrations above 22 mM have resulted in a decrease in cell viability (Johannson et al., 2002) and results suggest that more than 10 mM sodium glycocholate is harmful because of its high toxicity in lung tissues (Yamamoto et al., 1997). Nevertheless, bile salts may be useful in small amounts.

Administration of various unsaturated fatty acids (e.g., oleic, palmitoleic and linoleic acid) has been shown to have potent effects on salmon calcitonin absorption. Sorbitan trioleate, polyoxyethylene sorbitan monooleate and polyoxyethylene sorbitan trioleate only showed moderate enhancement, and enhancement by glycerol trioleate, ethyl oleate, oleyl alcohol, palmitic acid and stearic acid were relatively low (Hussain et al., 2004).

Citric acid has been used to modify the absorption of insulin and has been found to enhance and prolong insulin's hypoglycaemic effect. The hypothesis is that micelle formation by the lung surfactant might result in entrapment of the insulin, thereby preventing its absorption. Moreover, the presence of citric acid resulted in reduced monomer aggregation and enhanced absorption (Todo et al., 2001). Citric acid is considered a safe and effective absorption enhancer for pulmonary drug delivery.

Chitosan and trimethylchitosan, which are cationic polysaccharides, have also been widely used as absorption enhancers for proteins and peptides (Amidi et al., 2008; Grenha et al., 2008; Shoyele, 2008). A bioadhesive effect of chitosan particles was observed that may be useful in enhancing drug absorption following inhalation. In one study (Grenha et al., 2007), results indicated that chitosan did not adversely affect the viability of Calu-3 and A549 cells in an *in vitro* cell culture model of toxicity. However, another study has demonstrated inflammation-related biological effects from these particles on the rat lung. Inhaled chitosan microparticles induce a significant pulmonary inflammatory response in a dose-dependent manner after intratracheal administration. This effect was evident using a variety of tests including analyses of bronchoalveolar lavage fluid (for protein, lactate dehydrogenase activity and inflammatory polymorphonuclear) and analyses of lung tissue itself for leukocyte infiltration (direct cytological examination and myeloperoxidase activity). The pro-inflammatory effects of chitosan at doses between 2 and 10 mg/kg may be related to its high cationic charge (Huang et al., 2005). Other studies (Yang et al., 2007) have demonstrated that including chitosan may cause adverse effects on the chemical or physical stability of proteins.

On the other hand, studies on excipients normally used as drug absorption enhancers, such as sodium taurocholate, carnitine hydrochloride, dimethyl-β-CD or chitosan, have demonstrated that their use could improve aerosolization properties by modifying the particle morphology and surface of drugs (Ungaro et al., 2006). So, these excipients can be used not only to enhance the absorption of drug across the pulmonary epithelium but also to increase the deposition of drug in the lungs.

Although many of these excipients have been proved to be efficacious, none have satisfied the requirements for an ideal absorption enhancer in terms of toxicity. Nevertheless, the use of relatively non-toxic absorption enhancers in low concentrations such as sodium taurocholate, fatty acids and CDs for facilitating pulmonary absorption of peptides and proteins could be useful.

5.3.3.6. Biodegradable polymers. Controlled drug delivery systems have become increasingly attractive options for inhalation therapies. A large number of carrier systems have been developed and investigated as potential controlled drug delivery formulations to the lung, including drug-loaded lipid- and polymer-based particles (Zeng et al., 1995). As polymer degradation rates affect bioaccumulation, clearance of the biomaterial and its degradation products, the use of non-biodegradable polymers in the lungs should be prohibited. In addition, the safety of the degradation products must also be considered.

Biodegradable microspheres produced from natural and synthetic polymers (e.g., poly(lactic-co-glycolic) acid polymer (PLGA)) have been extensively investigated as drug carriers for administration via a number of different routes to ensure targeting and sustained drug release. PLGA microspheres have been used in pulmonary delivery for the controlled release of a wide range of drugs, including anti-asthmatic drugs, antibiotics, peptides and proteins. In comparison to liposomes, biodegradable microspheres may be more physicochemically stable both *in vitro* and *in vivo*. Thus, drugs entrapped in biodegradable microspheres may have a slower release rate and a longer duration of action than those incorporated within liposomes. The higher stability may enable biodegradable microspheres to be more easily formulated in a suitable pulmonary delivery device than liposome formulations (Zeng et al., 1995).

Among the various drug delivery systems considered for pulmonary application, nanoparticles demonstrate several advantages for the treatment of respiratory diseases, like prolonged drug release, cell specific targeted drug delivery and degradation within an acceptable period of time (Rytting et al., 2008).

However, due to its extremely slow rate of biodegradation, PLGA is considered unsuitable for pulmonary drug delivery, especially in cases where frequent dosing is required. After inhalation, production of the inflammatory cytokine IL-8 was significantly elevated in the case of PLGA and gelatin microparticles, which is indicative of their immunogenicity (Dailey et al., 2006; Sivadas et al., 2008). Moreover, the breakdown of PLGA leads to the acidic degradation products lactic and glycolic acid, which can irritate the lungs and cause DNA or peptide degradation and damage.

In summary, the choice of an excipient is a critical step in the development of new formulations for pulmonary administration. An excipient, by virtue of its nature, as well as the properties and ratio of its conjugate drug, can strongly modify the properties of drug particles and their aerodynamic behavior. Nevertheless, the number of excipients that have been declared safe for use for pulmonary delivery is restricted, and using a non-approved excipient involves extra workload, cost, time and carries the risk of rejection by regulatory authorities. Moreover, the addition of an excipient to a formulation, generally in the range of 60–99% (w/w), decreases

the dose of active drug that can be administered. This may cause difficulties in the case of drugs such as antibiotics that are active at relatively high doses or some peptides and proteins with low pulmonary bioavailability.

5.3.4. Carrier-free formulations

As previously discussed, to increase the flow and dispersal properties of micronized drugs, inhalation formulations are often composed of a mixture of excipient and active drug. Nevertheless, the choice of excipients authorized for inhalation is very limited. Moreover, the addition of an inert ingredient in elevated ranges decreases the dose of active drug administered. Most current DPIs can only deliver microgram quantities of drug rather than the milligram quantities needed for an inhaled antibiotic treatment. Thus, there is an increasing interest in formulations that do not require a carrier to aid the flowability of micronized drug; viz., "carrier-free" formulations. For example, sodium cromoglycate has been "pelletized" in weak and redispersible agglomerates without the need for any excipient (Edwards and Chambers, 1989). Some commercially available dry powder inhalers such as Pulmicort® and Bricanyl® have already employed carrier-free formulations even for low drug dosage such as budesonide and terbutaline, respectively. In this case, spheronization controls the aggregation of micronized particles. The aggregates are formed in rotating blenders, with the resulting large particle size providing the requisite flow properties or accurate dosing. The deaggregation of the agglomerates occurs by turbulent dispersion generated in the inhaler (Turbuhaler®), which is sufficient to overcome the interparticulate forces holding the micronized particles together. Nevertheless, the variability of dose emissions from such DPI systems has been found to be relatively high, with a total relative standard deviation of more than 15% around the average emitted dose.

Carrier-free tobramycin powders (Pilcer et al., 2009a) have been produced by spray drying in a one-step process, which is very useful for time saving purposes, industrial scale up and production. The idea was to modify the surface properties of the particles by coating the particles of drug with a homogeneously distributed continuous film of the active compound dissolved in a solvent system containing a mixture of different solvents, such as isopropanol and water. During nebulization of the suspension, droplets are composed of one or more particles in a solid state that are surrounded with solvent containing the dissolved drug. It is hypothesized that during the drying step, the dissolved particles form an amorphous coating film of the drug and allow an improvement of the aerodynamic behavior of the drug during inhalation. It is interesting to note that the presence of an amorphous coating markedly enhances the fine particle fraction, which was about 37% for raw tobramycin and approximately 57% for a powder obtained from a suspension containing 2% (v/v) water. Overall, this latter formulation was shown to retain its initial particle-size distribution and aerodynamic behavior after 12 months of storage at 40 °C and 75% RH. These new carrier-free formulations provide an attractive alternative for delivering high doses of antibiotics directly to the site of infection while minimizing systemic distribution (Pilcer et al., 2009a). Furthermore, the absence of excipients avoids the problem of long and costly safety studies and the risk of rejection by regulatory authorities.

6. Conclusion

To possess the optimal characteristics for efficient pulmonary delivery, formulations must be produced on the basis of numerous strategies that use a combination of particle technologies and excipients to develop the most desirable inhaled dosage forms.

In the particular case of dry powder products, the use of excipients has become a subject of active research. As the choices of excipients approved by the FDA for pulmonary administration is very limited, it should be noted that new excipients have to undergo stringent toxicity assessment, which would add uncertainty to the development of drug products. The dearth of regulatory guidance with respect to excipient safety, coupled with the extra workload, time and cost have slowed the development of these vital components of drug formulations.

A few sugars, such as trehalose or glucose, have been shown to be feasible alternatives to lactose or mannitol and will eventually find their way into worldwide approved products.

Use of excipients endogenous to the lungs, such as DPPC, DSPC and cholesterol, is interesting because they are biocompatible to the lung and can easily be metabolized or cleared by direct absorption through the epithelial cells or uptake by the alveolar macrophages. Nevertheless, a careful balance should be maintained between exogenous and endogenous components. Use of lipids for coating drug particles or incorporated in a matrix or in liposomes seems to be very promising in the future development of sustained release inhalation products. Moreover, their ability to modify the surface properties of particles through coating reduces the agglomeration tendency of a powder and increases the fine particle fraction.

Regarding other excipients widely used in other drug delivery routes, such as amino acids, surfactants or bile salts, although they provide an increase in the aerodynamic behavior of powders, they also have the ability to irritate or injure lungs, especially bile salts and surfactants. Nevertheless, they may be use with precaution because little is currently known about the local toxicity of various amino acids and their systemic absorption after pulmonary administration.

With regard to absorption enhancers, hydroxypropylated CDs still remain the most promising excipients considering primary safety data. To develop controlled release products, development of biodegradable polymers is suitable. In this case, the degradation rate of the biomaterial is of major importance to avoid bioaccumulation within the lungs. For this reason, the use of non-biodegradable polymers into the lungs should be prohibited.

The use of chitosan is also not advised as it has resulted in adverse biological effects and induced significant pulmonary inflammatory responses.

Therefore, different approaches to formulation that modify the surface properties of particles with the aim of minimizing the number and amount of additives used are very promising, allowing administration of highly dosed drugs and reducing possible adverse effects of excipients.

Therefore, in the development of new inhalation medications, consideration must be given to optimizing the formulation containing the drug substance to ensure a chemically stable and consistent dose, and to optimizing the design of the metering system within the inhaler itself to produce a convenient device that is comfortable and easy to use for the patient. Hence, the inhaler-drug combination is generally considered as a single medication, of which the *in vitro* performance and *in vivo* efficacy must be demonstrated.

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