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RESEARCH PAPER

Sustainable strategies for nano-in-micro particle engineering for pulmonary delivery

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Abstract With the increasing popularity and refinement of inhalation therapy, there has been a huge demand for the design and development of fine-tuned inhalable drug particles capable of assuring an efficient delivery to the lungs with optimal therapeutic outcomes. To cope with this demand, novel particle technologies have arisen over the last decade agreeing with the progress of pulmonary therapeutics that were commonly given by injection. Nanotechnology holds a considerable potential in the development of new release mechanisms of active ingredients to the deep lungs. For an accurate deep lung deposition and effective delivery of nanoparticles, respirable nano-in-micro formulations have been extensively investigated. Microparticles with nanoscale features can now be developed, and their functionalities have contributed to stabilize and improve the efficacy of the particulated dosage form. This paper reviews the different types of the aerosolizable nano-in-micro particles, as well as their sustainable production and characterization processes as dry powders. This review also intends to provide a critical insight of the current goals and technologies of particle engineering for the development of pulmonary drug delivery systems with a special emphasis on nano-micro dry powder formulations prepared by spray-drying and supercritical fluid-assisted techniques. The merits and limitations of these technologies are debated with reference to their appliance to specific drug and/or excipient materials. Finally, a list of most recent/ongoing clinical trials regarding pulmonary delivery of this type of formulation is described.

Keywords Inhalation · Dry powders · Spray drying · Supercritical-assisted atomization · Composite nanoparticles · Nanomedicine

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This article is part of the topical collection on Composite Nanoparticles

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Introduction

In the last decade, investigation on the interactions between nanoparticles and lung tissue has been strengthened, specifically in terms of potential health risk of particle inhalation. Notwithstanding, the huge advances in the nanotech field also brought new insights for novel delivery strategies, diagnosis, and therapeutic

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approaches through the inhalative route (Geiser et al. 2013). With rapid advances in nanotechnology, the use of drug nanoparticles has become a subject of a very active research, and it has triggered the interest in the lungs as a major route of drug delivery for both systemic and local treatment (Hu et al. 2013). Nanoparticles have been foreseen as an efficient way to develop controlled release delivery systems for the lungs. There are many types of nanoparticulated systems being explored for lung delivery. In fact, nanoparticle systems can be engineered to exhibit several desirable features for diagnosis and therapy (theragnosis) of specific conditions. Despite their ability to protect therapeutics at both extracellular and intracellular levels and then release them in a sustained and controlled manner, they are still able to penetrate into deep tissue and then suffer cellular uptake, due to their nanometric scale. Nanoparticles functionalization also provides a stealth surface to prevent opsonization, therefore, increasing their residence time and enhancing therapeutics delivery at the accurate site of action. Nano-based theranostics offer a pathway for personalized medicine allowing real-time diagnosis of lung inflammatory state and carrying the optimal therapy for individual patients with reduction of the undesirable drug side effects. Innovative design of nanoparticles allows for the assimilation of several functions, such as cell targeting, ultra sensitivity imaging, and therapy, all into one system. Surface-functionalized gold nanoparticles, multifunctional-quantum dot conjugated, liposomes, and multi-layered assemblies have been extensively exploited to face the urgent need to further develop novel multifunctional nanosystem theragnosis of lung complications (Sivadas et al. 2008; Akiyama et al. 2009; Lai et al. 2010; Kurmi et al. 2010; Restani et al. 2014). The combination of nanotechnology and the pulmonary route for administration of aerosolizable drugs brings out new and exciting breakthroughs for the diagnosis and therapy of several types of lung and systemic diseases. Such type of delivery to the lung (air-to-lung) and via the lung (air-to-blood) can circumvent the lack of effective methods via other routes of administration. However, nanoparticles are in a size range which is not suitable for deep lung delivery and can be easily exhaled or mucociliary cleared out before reaching the underlying epithelia (Lai et al. 2010; Hu et al. 2013). Moreover, nanoparticles' nature poses noteworthy challenges in powders development. Their increased surface area results in high Gibbs free energy, which may lead to particles agglomeration and consequent formulation instability. Therefore, the major challenge for pulmonary delivery of nanoparticles is to find a proper carrier system. Increased effectiveness of an inhalation treatment may be achieved by the quick dissolution of powder particles in the airway mucus and rapid diffusion to the destination tissue. Hence, a novel-particulate form incorporating nanoparticles into micro-scale structures has been engineered to overcome the issues of storing and delivering the drugs and other biomolecules to the lungs. Aerosols for inhalation therapies may be produced by pneumatic ultrasonic nebulizers, pressured metered dose inhalers (pMDI), and dry powder inhalers (DPI) (Odziomek et al. 2012). From all the formulations available for inhalation, dry powders are usually preferred as they exhibit the most suitable behavior for pulmonary delivery such as stability and bioavailability of active ingredients, when compared to their aqueous counterparts (Hardy and Chadwick 2000; Al-Qadi et al. 2012). Moreover, storage and distribution in a cool-chain are not necessary as the usage of a DPI provides longer storage stability to the powders therein (Klingler et al. 2009). In DPI formulations, drug nanoparticles have been blended with carrier materials, producing nano-in-micro formulations like Trojan and Strawberry particles or particles with nanofeatures. In DPI, the lower density of such nanoparticles agglomerates results in smaller mass median aerodynamic diameter (MMAD) and consequently increases lung deposition and powder flowability. Moreover, nanoparticles in inhaled therapeutics have also been demonstrating an increased residence time in the lung muco adhesive surfaces and reduced mucociliary clearance (Zhang et al. 2011).

Although microparticles can be manufactured by many different processing methods, this review focuses mainly on spray-drying and supercritical fluids technology in the production of totally "green" dry powders formulations. Wet chemistry, phase separation processes, and other drying methods, such as spray freeze drying, have also been widely used for particle engineering purposes and have already been reviewed elsewhere (Chow et al. 2007; Mohajel et al. 2012).

The lung as a delivery target for nanomaterials: nano-delivery via aerosol

Lungs, skin, and intestinal tract are in direct contact with the environment. Therefore, these organs are

likely to be a first port of entry for nanomaterials into the body. In fact, epidemiological studies regarding the exposure of the body to atmospheric particles showed a positive correlation between the increase of such contact and the short-term increase in morbidity and mortality (Borm and Kreyling 2004; Powell and Kanarek 2006). Since inhalation is by far, the most significant exposure route for air-borne nanoparticles, the number of studies regarding on nanoparticles deposition along the respiratory tract has been increasing. Bearing this knowledge in mind, the systemic administration of proteins or other macromolecules is being surpassed by the delivery of such molecules through the pulmonary inhalation route (Hoet et al. 2004; Oberdörster et al. 2005; Amidi et al. 2008; Tolman and Williams 2010; Zhang et al. 2011; Kaur et al. 2012; Wanakule et al. 2012; Hu et al. 2013). Actually, needle-free drug delivery systems have been receiving increased attention as a noninvasive alternative owing to the unique physiological features of the lungs (Adami et al. 2011). Ones are characterized by large absorptive surface area (>100 m^2), thin blood-barrier membrane (0.1–0.2 μm), elevated vascularization, and low enzymatic activity which, together, facilitates macromolecule transport into systemic circulation, leading to very rapid action and high bioavailability. Furthermore, drug and other molecules delivery through lungs are not subjected to hepatic first-pass effect (Pulliam et al. 2007; Amidi et al. 2008; Zhang et al. 2011; Al-Qadi et al. 2012; Hu et al. 2013). Moreover, the favorable physiological environment of the deep lungs, such as physiological pH and reduced mucociliary action, overcomes many problems of other non-invasive vaccine targets, like rapid clearance, poor absorption, exposure to digestive enzymes, and the antigenic tolerance (Pulliam et al. 2007). So far, several protein-based drugs, such as insulin, human growth hormone, calcitonin, and deslorelin, have been reported to reach the systemic circulation following aerosol administration. Hence, pulmonary delivery holds a great potential for the treatment of several respiratory diseases like asthma, tuberculosis, influenza, cystic fibrosis, chronic obstructive pulmonary diseases (COPD) and lung cancer. Actually, recent studies regarding cancer immunotherapy using genetically engineered T-cells to target cancer cells have shown remarkable results. With more people living beyond age 65, cancer's incidence is projected to rise even more in the coming years. Therefore, the population who might benefit from immunotherapy is potentially quite large. By combining this knowledge with pulmonary delivery, extraordinary outcomes may arise. First pass metabolism may be avoided, and cancer, especially non-small lung cancer (one of the leading causes of death worldwide), may be effectively treated (Couzin-Frankel 2013).

Table 1 Mechanism of aerosol deposition (Chow et al. 2007; Yang et al. 2008)

Site	Particle size (μm)	Mechanism
Large conducting airways	>5	Inertial impaction
Respirable airways		
Smaller airways	3–5	Deposition
Alveolar airspaces	1–2	Deposition
	<0.5	Exhalation

Localized drug delivery not only shows a great promise in the treatment of such diseases but also reduces the systemic toxicity (Kaur et al. 2012). Although advantageous, efficient pulmonary delivery and effective delivery of biologic drugs inside cells of the deep lung are also very challenging missions (Hu et al. 2013). In fact, particle deposition and retention in the different pulmonary stages are determined by three main factors: respiratory tract anatomy, breathing pattern, and particle size (Geiser et al. 2013). Although some conflicts regarding the range for optimal aerodynamic particle diameter (d_{ae}) to efficiently reach the deep lungs may exist, the most recent works state that the aerodynamic diameter of aerosol particles should be between 0.5 and 5 μm. Smaller particles may fail to deposit and are exhaled, whereas bigger ones may accumulate in the mouth and throat (Choi et al. 2010; Wanakule et al. 2012; Hu et al. 2013). Moreover, smaller particles are more likely phagocytized and have the tendency to aggregate due to van der Waals and electrostatic forces (Li et al. 2010) making large porous particles with low density suitable for lung delivery (Yang et al. 2009). When the MMAD is more than 5 μm, inertial impaction exists in the oropharynx and large conducting airways, while the smaller airways are reached when the aerodynamic diameter is smaller than 5 μm (Chow et al. 2007; Yang et al. 2008). Table 1 describes the mechanism of dry powder deposition along the respiratory tract.

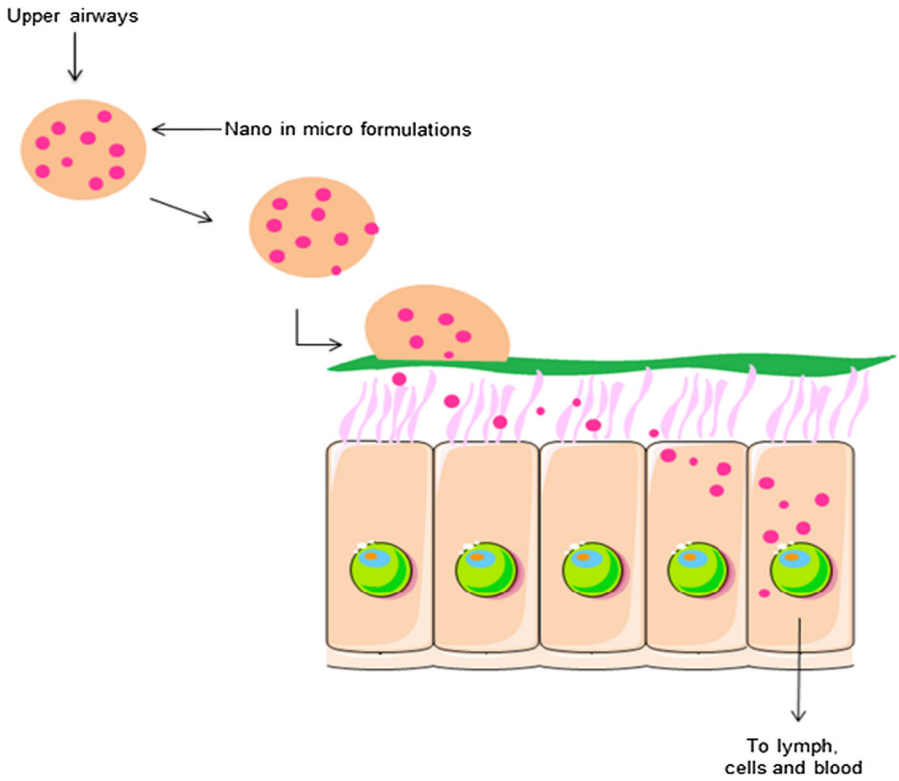

Fig. 1 Mechanism of nanoparticle delivery through aerosol of nano-in-micro formulations. Once in the lung epithelia, microcarriers must readily dissociate into the primary nanoparticles in an aqueous medium without significant destruction of the delivery advantages associated with the nanoparticulate systems. Then, nanocarriers may travel to their site of action, due to specific targeting exhibited in nanoparticles surface, and exert their theragnosis effect

However, drugs and other biomolecules that are targeted to intracellular machinery, generally require nanocarriers to improve mucus diffusion and cellular uptake by the epithelial cells. These paradoxical requisites turn complex the development of an efficient carrier system: the particles should comprise sizes between 0.5 and 5 μm during the inhalation, then swell and increase their size upon lung settling evading the macrophage clearance in the deep lung and enabling the release of smaller particles that penetrate lung mucus and deliver drugs, proteins, or other biomolecules intracellularly, minimizing non-specific side effect to the healthy tissue, as displayed in Fig. 1 (Wanakule et al. 2012; Sadhukha et al. 2013). For suitable cellular internalization, nanoparticles should not exhibit sizes larger than 150 nm (Duncan et al. 2010). Notwithstanding, particles comprising sizes ranging between 20 and 50 nm have shown to have higher cellular internalization (Oishi et al. 2009; Dreaden et al. 2012). Moreover, it has already been proved that if smaller nanoparticles (hydrodynamic diameter (HD) less than $\approx$ 34 nm and a noncationic surface charge) are used, specifically in pulmonary delivery, nanoparticles can rapidly translocate from the lung epithelium to mediastinal lymph nodes. This should provide high levels of drug to pulmonary lymph nodes, which could be used to deliver antibiotics and anti-inflammatory drugs to treat other lung infections, tumor metastases, and inflammatory conditions (Choi et al. 2010).

Table 2 Different types of inhalers

Type of inhaler	Advantages	Disadvantages
Nebulizers	Alveolar deposition can be easily achieved	Limited portability Difficult to use
pMDI	Easy portable Tamper Proof Multidose	Difficult to use Use of propellants
DPI	Propellant-free Easy to use Formulation stability	Complex development and manufacture Dose uniformity problems

Advantages and disadvantages (Telko and Dsc 2005; Packhaeuser et al. 2009; Stegemann et al. 2013)

Table 3 Examples of marketed inhalers (Gabrio et al. 1999; Pilcer and Amighi 2010)

Type of inhaler								
Nebulizers		pMDI			DPI			
		Press-and-breath products		Breath-actuated products	Single Dose	Multiple Unit Dose	Multidose	
		Bronchodi-lators	Steroids					
Atrovent	Bisolvon	Maxair®	Beclazone™	AeroBec™ 100 Autohaler™	Spinhaler®	Diskhaler®	Turbuhaler®	
Duovent	Beclojet	Proventil®	Beclovent®	Beclazone™ 100	Rota-haler®	Diskus®	Easyhaler®	
Duo-Medihaler	Flixotide	Sultanol	Becotide ™ 100	Maxair ™ Autohaler™	Aerolizer™	Aerohaler®	Novolizer®	
Ventolin	Lomudal	Ventolin®	Flixotide™	QVAR ™ Autohaler	Inhalator®	Flowcaps®	Clickhaler®	
Pulmicort			Pulmicort		Eclipse	MicroDose DPI	Pulvinal®	
					Turbo-spin	Delsys DPI	Ultrahaler®	
					AIRTM Inhaler	Techno-haler®	Taifun®	

Devices for pulmonary inhalation—dry powder inhalers

Lung delivery of nano and micro formulations may rely on either dry solid particles as in DPIs, suspensions of nanoparticles as in nebulizers and pMDIs, or the formation of nanodroplets from solution (Rogueda and Traini 2007; Zhang et al. 2011). Table 2 shows a comparison between the different types of existing inhalers.

Pulmonary drug delivery of controlled release formulations may provide an effective approach to orally deliver antibiotics for clearing persistent lung infections. From all the formulations available for inhalation, dry powders are usually preferred as they exhibit the most suitable behavior for pulmonary delivery such as stability and bioavailability of active ingredients, when compared to their aqueous counterparts (Hardy and Chadwick 2000; Al-Qadi et al. 2012). Moreover, storage and distribution in a cool-chain are not necessary as the usage of a DPI provides longer storage stability to the powders therein (Klingler et al. 2009).

Although the general reviews do allude to the use of different inhaler devices, we here narrow the focus to the recent studies regarding DPIs. For the sake of simplicity, the term nanoparticles will be used to cover either solid nanoparticles or other biomolecules at the nanoscale. Thus, the term "nano" refers to all particulates, droplets, or solid particles for which at least one dimension is 1–1,000 nm. DPIs are at the forefront in the pulmonary delivery technology. They overcome problems such as desynchronized dose discharge during the inhalation process as they are breath actuated (Malcolmson and Embleton 1998; Hardy and Chadwick 2000). DPIs are also suitable for delivering a broad range of drugs than pMDIs (which used to be the leading application form of inhalation but were surpassed by DPIs as they require difficult hand–lung coordination by the patient and use environmentally damaging CFC propellants). Moreover, DPIs can deliver a range of doses from some μg to more than 20 mg via a single inhalation (Malcolmson and Embleton 1998). Besides listing the marketed nebulizers and pMDIs, Table 3 also displays several types of DPIs that can be classified as "single-dose" devices, where a single dose is provided in a capsule; "multiple unit dose" devices, which contains a small amount of doses in capsules or blisters; or

"multidose" devices, where the powder is stored in a reservoir, and the doses are metered (Newman and Wilding 1998; Malcolmson and Embleton 1998). The working principle of a DPI is to deliver a prescribed dose of powder aerosol into air inhaled by the patient during a single breath in a process designated "fluidization," preventing the powder from air ambient exposure. The vast majorities of fine drug particles are micronized drug blended with large carrier particles which enhance flow, reduce aggregation, and assist in dispersion (Kaur et al. 2012). Pharmaceutical products to be used in the DPI may result in high local levels of drug in epithelial fluid of the airways and lower respiratory tract (Amidi et al. 2008). However, the application of drug nanoparticles for pulmonary delivery in DPIs is not straightforward, as it was already explained, since the direct inhalation of drug nanoparticles is not viable due to their easy aggregation. To overcome this problem, spray-drying and, more recently, supercritical fluid-assisted atomization techniques have been used to manufacture large composite porous particles or hollow particles composed of nanoparticles aggregates, to be then delivered to the lungs using DPIs (Hu et al. 2013).

Particle engineering

Spray-drying technique

Spray-drying technique has been extensively applied in the production of dry powder formulations due to its simplicity, scale-up, ease of operation, and the ability to produce composite materials. In 2010, spray drying was considered as one of the most interesting technologies used in the pharmaceutical field (Gil et al. 2010). Appealing to this technology, powders/particles properties such as size, morphology, density and level of residual solvent, can be manipulated. This remarkable feature has led to its application in the formulation of a wide variety of powders or advanced solid forms. A wide range of products may be obtained, mainly very fine powders for inhalation to large particles for direct compression, solid dispersions for enhanced bioavailability, and microcapsules for drug shelter and/or controlled release (Gil et al. 2010). However, spray dryers scale-up have to be strategically design so that the quality of the resultant materials is maintained, and no considerable and expensive losses are exhibited (Gil et al. 2010; Son et al. 2013). Spray-drying technology is a one step process that comprises the atomization of the feed solution into a spray. The solvent in the atomized droplet is thermally removed in the spray-drying chamber leading to the particles drying, which are then separated from the air through a cyclone or a filter bag. Typically, polymeric- or lipid-based materials are used as carriers to prepare drug-loaded nanoparticles in the form of suspension, followed by spray drying under specific conditions in order to form large porous nanoparticle aggregates with spherical geometry and rough surfaces. However, such technique has some disadvantages including the possible degradation of heat sensitive fine particles and inefficient yields (Dunbar et al. 1998; Malcolmson and Embleton 1998; Millqvist-fureby and Malmsten 1999; Alderborn et al. 2003; Elversson and Millqvist-Fureby 2005; Ozeki et al. 2006; Chow et al. 2007; Zhang et al. 2011; Kaur et al. 2012). Spray-drying technique has been employed for decades in the manufacture of drug microparticles for inhalation therapy. Recently, Jun and colleagues produced micron-sized spherical agglomerates of pure sodium cromoglycate to improve aerosolization performance in dry powder inhalers. Such achievement was accomplished using combined technologies as liquid anti-solvent precipitation followed by instant agglomeration of nanoparticles into porous spherical microparticles by spray-drying technique. The authors were able to increase in 50 % in vitro aerosol deposition in comparison with the control samples (Li and Birchall 2006; Hu et al. 2013).

Supercritical fluid-assisted processes

Supercritical fluid-assisted processes have lately emerged as green and innovative alternatives for a wide variety of processes such as: solubility enhancement of poorly soluble drugs, plasticization of polymers, surface modification, nanosizing and nanocrystal adjustment, and chromatographic extraction. An increasing interest regarding this research area has been triggered by the numerous advantages that this technology offers over the conventional methods (Girotra et al. 2013). Supercritical fluids (SCFs) are defined as compressed gases or liquids above their critical pressures and temperatures. SCFs possess central advantages as solvents or antisolvents for

pharmaceutical manufacturing. Due to its low critical temperature (31.1 °C), moderate pressure (73.8 bar), inert and environmentally friendly nature, non-flammability, and low cost, carbon dioxide is the most used fluid under supercritical conditions ($scCO_2$) for pharmaceutical approaches. One of the most extraordinary properties of $scCO_2$ in processing nanoparticles is its tunable solvent power that facilitates the extraction and separation of organic solvents allowing the production of pure and dry particles or as a pure aqueous suspension while enabling a clean and recyclable precipitation process at very low temperatures. Due to these extensive properties, applications using SCFs, particularly CO_2, have been extensively investigated for respiratory delivery applications (Amidi et al. 2008; Zhang et al. 2011). There are two main conventional methods for obtaining microparticles using $scCO_2$: the rapid expansion of supercritical solutions (RESS), where the solid material is dissolved in CO_2 under supercritical conditions, and then the solution is rapidly expanded by lowering the pressure, promoting a rapid cooling rate and inducing supersaturation leading to microparticle formation (Türk 1999; Reverchon and Adami 2006; Martín and Cocero 2008; Sanli et al. 2011; Girotra et al. 2013); and supercritical antisolvent (SAS), where particles are produced when a solution is brought into contact with $scCO_2$ in a semi-continuous method. This process has the advantage of controlling the physical form of powders by varying the working conditions of temperature, pressure, or solution flow rate. However, solvents used in this method must be completely miscible in $scCO_2$, which may pose a problem when aqueous solvents are used, since water is not miscible with $scCO_2$ (Malcolmson and Embleton 1998; Reverchon 1999; Chow et al. 2007; Martín and Cocero 2008; Girotra et al. 2013). Particles from Gas Saturated Solutions (PGSS) are a $scCO_2$-assisted process based in two main steps, the first being the saturation of a solute-containing solution with CO_2 in a static mixer, and the second one the mixture's expansion through a nozzle into a spray tower. The CO_2 expansion from the resulting droplets leads to an atomization, which also happens due to the fast temperature and pressure reduction (Joule–Thomson effect) (Martín and Weidner 2010; Martín et al. 2010). Particle formation via polymerization-induced phase separation (PIPS) is a process that produces particles by means of a synthesis in a supercritical fluid medium, such as polymerization (Yeo and Kiran 2005).

Table 4 Spray drying versus $scCO_2$ (Chattopadhyay and Gupta 2001; Zhang et al. 2011)

Technique	Advantages	Disadvantages
Spray-drying	Furthest optimized On-step process to produce dry powder Control of particles' properties Potential for producing antigenic nanoparticles in-line with the aerosols	Production of a broad size distribution Possible degradation of heat sensitive drugs Inefficient yields
Supercritical CO_2 - assisted processes	Use of mild conditions Efficient solvent extraction Clean and environmentally friendly Benefic physical properties of the supercritical fluids Narrow particle distribution High yields Produces successful drug nanoparticles	Expensive equipment High critical pressure High pressure and associated hazards

Moreover, supercritical fluid extraction of emulsions (SFEE) uses $scCO_2$ to extract the organic phase out of emulsions. Each emulsion droplet allows precipitation, encapsulation, or blending resulting in uniform particles (Chow et al. 2007). More recently, the supercritical-assisted atomization (SAA), a process based on the solubilization of controlled quantities of CO_2 into the liquid solution has proved to have many advantages over conventional methods. It offers the possibility of operating in a continuous mode in mild operating conditions and the ability to use both organic and inorganic solvents while providing a good control over particle size and distribution (Reverchon 2002, 2007; Reverchon et al. 2006; Adami et al. 2009; Casimiro et al. 2011; Cabral 2013). Table 4 displays the advantages and disadvantages of spray-drying technique and supercritical CO_2-assisted processes.

Design of nano-in-micro dry powder formulations

As already mentioned, nanoparticles are unique carrier platforms of proteins and other macromolecules for

Table 5 Nano in micro formulations prepared by conventional spray drying or supercritical assisted atomization processes

Process	Type	Drug	Excipients	Particle size (μm)
Spray-drying	Trojan particles	n.a.	DPPC-DMPE-lactose	4–8 (Tsapis et al. 2002)
		Rifampicin	PLGA/L-leucine	4.2 (Sung et al. 2009)
		Octreotide acetate	n.a.	5.68 (Yang et al. 2013)
	Particles with nanofeatures	Human IgG	PLGA/lactose	4.2 (Kaye et al. 2009)
		Clarythromycin	PLGA/L-leucine	5.3-8.9 (Moghaddam et al. 2013)
		Thymopentin	Mannitol/L-leucine	4.1 (Li et al. 2010)
	Strawberry particles	β-galactosidase	Lactose	226 (Genina et al. 2010)
Supercritical CO_2	Particles with nanofeatures	Magnetite nanoparticles	PMMA/PLGA	0.97-1.55 (Chattopadhyay and Gupta 2002)
		Terbutaline sulfate	Lactose	3.2 – 3.4 (Rehman et al. 2004)
		Deslorelin-HPβCD	PLGA	2.2/13.8 (Koushik et al. 2004)
		pDNA	Chitosan	12.2-13.2 (Okamoto et al. 2003)
		Lysozyme; doxorubicin	PLGA	2.17–18.2 (Yang et al. 2009)

n.a. not available

pulmonary delivery. The capacity of nanoparticles to penetrate into intracellular compartments and the possibility of avoiding macrophages' phagocytosis increases the carrier's residence time in the lung allowing a sustained drug release (Al-Qadi et al. 2012; Ungaro et al. 2012). Moreover, reports have been showing that patients prefer the DPIs delivery system to either the nebulizer or intravenous administration in a single-dose unit, demonstrating an improvement in patient compliance to adhere to DPIs (Crowther Labiris et al. 1999).

For DPI formulation, nanoparticles have been designed to reduce the interparticle attraction forces and to improve the DPI blending with carrier materials, forming Trojan, strawberry and nanoparticles aggregates (particles with nanofeatures) (Rogueda and Traini 2007). To be therapeutically effective, microparticles must readily dissociate into the primary nanoparticles in an aqueous medium without significant destruction on the delivery advantages associated with the nanoparticulate systems (Li et al. 2010). Inhalable powders have to fulfill certain requirements; the increase of the respirable fraction with a decreasing average particle diameter is a fundamental matter of fact (Hickey et al. 2007). The general assumption is that particles smaller than 5 μm are not deposited in the upper airways but are able to penetrate into the deep lung. Systemically effective drugs, which have to attain the alveoli, should even have a mass median aerodynamic diameter less than 2 μm (Klingler et al. 2009). To overcome these issues, the nanoparticles can be microencapsulated in order to improve their aerodynamic properties and stability and so, produce suitable lung delivery particles. Table 5 and Fig. 2 display the most promising nano-in-microparticles already developed.

Trojan particles

Trojan particles are micron-sized large porous particles (LPPS) that get deposit in the lungs thanks to their aerosolization and physical properties and then dissociate to the primary nanoparticles, avoiding phagocytosis and taking advantage of its drug delivery properties. Tsapis and coworkers produced large porous nanoparticles composed of dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylethanolamine (DMPE), and lactose using spray drying, which allow the control of the drying time of the droplet avoiding nanoparticle diffusion. By measuring the geometric and aerodynamic diameter, they noticed that the LPPs properties and the FPF improved when the nanoparticles were encapsulated (Tsapis et al. 2002).

Sung and coworkers formulated a dry powder for the treatment of tuberculosis by encapsulating rifampicin in poly (lactic-co-glycol acid) (PLGA) nanoparticles and then spray dried into porous nanoparticle-

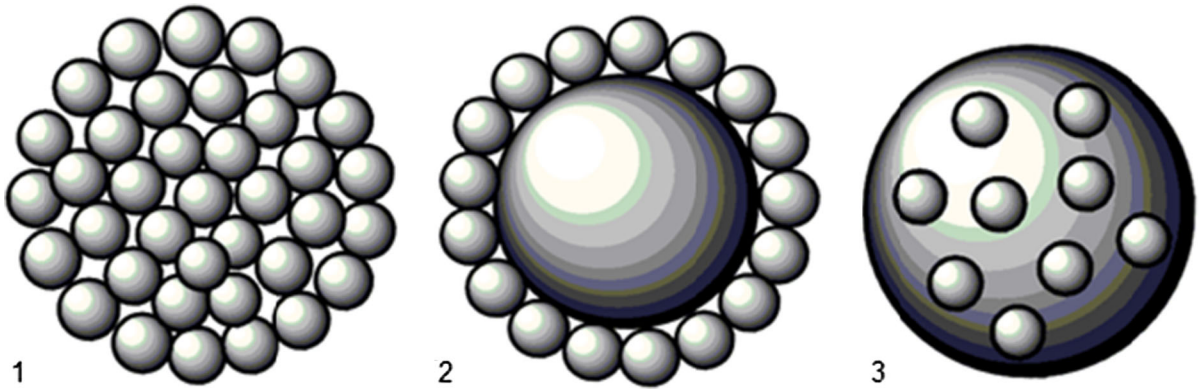

Fig. 2 Nanoparticle-based dry powders for inhalation: **1** Trojan particles; **2** Strawberry particles; **3** Particles with nanofeatures

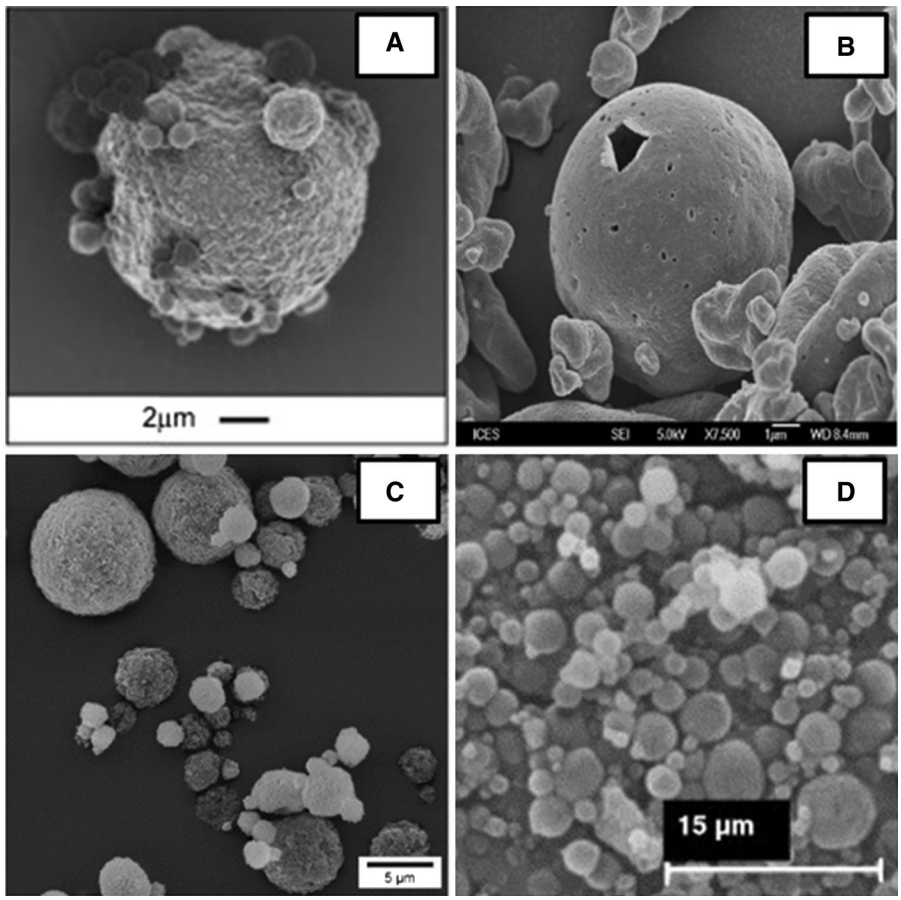

Fig. 3 SEM pictures of a representative panel of Trojan microparticles reported in literature: **a** Reproduced with the permission of Elsevier (Gómez-Gaete et al. 2008): spray dried microparticles prepared with DPPC, hyaluronic acid (HA) and PLGA nanoparticles; **b** Reproduced with the permission of Elsevier (Hadinoto et al. 2007): spray dried microparticles prepared with DPPC and PMMA-methoxy(polyethylene glycol)methacrylate (MeOPEGMa) nanoparticles; **c** Reproduced with the permission of Elsevier (Al-Qadi et al. 2012): spray dried microparticles prepared with mannitol and chitosan/TPP nanoparticles; **d** Reproduced with the permission of Elsevier (Tewa-Tagne et al. 2006): microparticles prepared with colloidal silicon dioxide as drying auxiliary and oily-core PCL nanocapsules

aggregate particle (PNAPs) with L-leucine. Experiments showed that the particles have both systemic deliver and long levels of drug for up to 8 h (Sung et al. 2009).

More recently, the spray-freeze drying method was used to produce PNAPs containing a model peptide, octreotide acetate. When peptides are used in the process, temperature becomes a concern, which made this group to deviate from the conventional spray-drying process to keep the model peptide active. The in vivo experiments showed higher levels of plasma aspartate amino-transferase when in comparison with delivery through subcutaneous injection. To improve the encapsulation efficacy and the drug loading, hydrophobic ion pair complexes were used (Yang et al. 2013). Some examples of SEM images of Trojan microparticles are depicted in Fig. 3.

Particles with nanofeatures

Even though Trojan particles have shown some success, there are some drawbacks when it comes to the preservation of the particles integrity and their aerodynamic properties due to drying process of the NPs suspension. Another type of particles produced is the nano-embedded microparticles that consist of micro-encapsulated drug-loaded NPs, which excipient is degraded when they reach the deep lung, releasing the nanoparticles (Ungaro et al. 2012).

Lately, polymeric nanoparticles production has been growing once its effectiveness in sustained release, and high drug capacity has been shown. One of the most used polymers is PLGA because of its biodegradability and biocompatibility, and also for its long clinical experience (Makadia and Siegel 2011). Besides, it is used in a host of Food and Drug Administration (FDA)-approved therapeutic devices and has a long safety record (Cruz et al. 2010). Another common polymer is chitosan, a polysaccharide biopolymer which has a high interest due to its low toxicity, biocompatibility, biodegradability, and mucoadhesive properties (Al-Qadi et al. 2012; Sinsuebpol et al. 2013). As for the inert excipients, sugars are the most used ones, such as lactose and mannitol, because of the stability they provide for the spray-dried particles (Grenha et al. 2008). Mannitol is said to be advantageous comparing to lactose, because it has less hygroscopicity, no reducing effect and it is also nontoxic (Li et al. 2010; Sinsuebpol et al. 2013). On the other hand, lactose is the only FDA-approved sugar carrier for dry powder aerosol formulations (Mansour et al. 2009). Some nano-in-microparticles were developed by co-spray-drying the nanoparticles with bulking agents and dispersibility enhancers, such as L-leucine, a nontoxic and nonirritant amino acid, known to improve flowability and dispersibility (Vehring 2008; Rowe et al. 2009). For instance, Kaye and colleagues produced nano-in-micro particles aimed for antibody delivery in the lungs by SIMANIM (Simultaneously Manufactured Nano-in-Micro) (Kaye et al. 2009). A double-emulsion containing human IgG, lactose, PLGA and DPPC was spray-dried, leading to lactose microparticles loaded with PLGA nanoparticles, where the antibody was released for 35 days in a pH 2.5 media and was stable and active. With the addition of L-leucine, the fine particle fraction (FPF) was improved from 37 to 52 %, which was also concluded by Moghaddam and co-workers that spray-dried Clarithromycin-encapsulated PLGA nanoparticles with L-leucine to treat lung infections such as pseudomonas aeruginosa (Moghaddam et al. 2013). The resulting micro-carriers had diameters between 5.3 and 8.9 μm, and the presence of L-leucine showed improvements in yield and in FPF since it reduced the particles cohesiveness. Li and coworkers produced thymopentin-loaded solid lipid nanoparticles encapsulated in mannitol/leucine microparticles. From in vitro and in vivo studies, it was shown that the mannitol provided protection to the peptide, which had a strong therapeutic effect, since the layer formed around the nanoparticles avoids lipids coalescence. Also the moisture sorption and surface tension diminished thanks to the presence of L-leucine (Li et al. 2010).

Al-Qadi and colleagues developed microencapsulated chitosan nanoparticles for pulmonary protein delivery, by co-spray-drying them with mannitol. The resulting microspheres have a mean diameter of 2.5 and 2.8 μm, and quantitative analyses of hypoglycemic effect and lung distribution studies showed that this delivery system gets to the deep lung and releases insulin in its bioactive form (Al-Qadi et al. 2012). Even though spray-drying is the conventional technique to produce dry powders, the use of supercritical CO_2 has been growing over the years. An example is the supercritical antisolvent (SAS) technique, used by Chattopadhyay and collaborators to coprecipitate poly(methyl methacrylate) (PMMA), PLGA, and

Eudragit RS with a suspension of magnetite nanoparticles. This magnetic property of the particles allows a specific targeting due to the attractive forces with the magnetic field applied to the target site. It was observed a slight difference in morphology when the magnetite nanoparticles were added, because the particles showed a roughened surface (Chattopadhyay and Gupta 2002).

Strawberry particles

This particle designation first appeared during the 2005 Aerosol Society meeting, and it is associated with micro-carriers coated with active nanoparticles connected via physical absorption (Rogueda and Traini 2007). This formulation is advantageous because the use of micronized drug blended with larger carrier helps flow and prevents aggregation (Telko and Dsc 2005). Once the coated nanoparticles work as spacers between the microparticles, the adhesion between them is diminished, making the handling easier. The coating process can be done by electrostatic forces, where the different size particles have opposite charges, avoiding aggregation between themselves and only with each other (Linsenbühler and Wirth 2005). There are also capillary and van der Waals forces and mechanical interlocking responsible for particle–particle and particle–surface interactions. Usually, lactose particles are the chosen carrier because, besides all the advantages already mentioned, their large size, when the airflow is introduced, allows the generation of primary drug particles that can then be carried until the deep lungs while the carrier impacts in the oropharynx and is cleared (Telko and Dsc 2005; Hickey et al. 2007). It is also possible to nano-coat proteins in micro-carriers using an ultrasound technique. Genina and associates used β-galactosidase and lactose to produce appropriated pharmaceutical formulations for lactose intolerant patients and measured the proteins quantity in the surface by its enzymatic reaction product, D-galactose, with results between 0.2 and 5.7 mg/g of lactose (Genina et al. 2010).

Characterization of inhaled particles

Upon testing a formulation for future lung delivery, there are several parameters that should be confirmed, such as its safety, efficacy, and quality (Storey and Ingvar 2011). To address these features, there are specific characterizations that need to be carefully addressed. In this review, the most important evaluations are described. One of the most important features is the particle size diameter, which must be known to evaluate the aerodynamic diameter (d_{ae}). The d_{ae} of a particle is defined as "the diameter of a sphere with an unit density that has the same terminal settling velocity in still air as the particle in consideration" and is represented by the following equation:

$$d_{ae} = d_{geo}\sqrt{\frac{\rho_p}{\rho_o x}} \quad (1)$$

where ρ_p is the particle mass density, ρ_o is the unit density, and x is the particle dynamic shape factor. Such factor is also defined as the ratio of the drag force of the particle to that of a sphere of equivalent volume. From this equation, it is possible to verify that the $d_{ae.}$ can be reduced by decreasing particle size or density, or by increasing the dynamic shape factor (Pilcer and Amighi 2010). Both size and distribution can be determined with the use of automated microscopy and image analysis and methods that use geometric features and physicochemical properties (Telko and Dsc 2005; Vehring 2008). In the other hand, by means of an in vitro aerosolization study using a multistage cascade impactor, it is possible to obtain the MMAD, which is determined as the particle diameter corresponding to 50 % of the cumulative distribution and to find the fraction of emitted dose that reaches the deep lung. The fine particle fraction (FPF) is the mass of particles under a certain cut-off diameter, usually around 5 μm, with reference to the emitted dose, i.e., the particles proportion to exit the inhaler and which can also be determined using a Dosage Unit Sampling Apparatus (DUSA) (Chow et al. 2007; Sivadas et al. 2008).

The selection of the optimal solid form is a crucial aspect upon the development of pharmaceutical compounds, due to their ability to exist in more than one form or crystal structure (polymorphism). These polymorphs show different physical properties which affect their biopharmaceutical properties (Storey and Ingvar 2011). Solid state analyses are often used to guarantee both safety and efficacy of drugs or pharmaceutical compounds and to validate the control of the pharmaceutical manufacture process (Stephenson et al. 2001). X-ray power diffraction is one of the

most used techniques to attest the solid state of a formulation, and it is used to define the intermolecular spacing of the unit cell, which defines a crystalline system. For lung delivery applications, it is important to have an amorphous carrier, not only for storage proposes, but because it also increases dissolution rate, and it has high water adsorption capacity. In the other hand, the active pharmaceutical ingredient (API) is usually preferred in a crystalline form, since it maintains the bioavailability of the drug, maximizing its efficiency and minimizing the required dosage (Telko and Dsc 2005; Pasquali et al. 2006; Hickey et al. 2007; Chow et al. 2007).

The dry powders' properties and performance in the lungs can be determined by in vitro studies that can match the regulatory body criteria, even though in vivo events such as lung deposition and pulmonary pharmacokinetics are difficult to experimentally test. The in vitro experiments for inhalation particles use lung epithelial cell models which have the advantages of being simpler, more controllable and cheaper than the in vivo approach. The main goal is to determine the transepithelial transport kinetics of test molecules in order to compare it with in vivo lung absorption (Sakagami 2006). However, cell cultures used in in vitro studies do not provide an accurate correlation with in vivo absorption and also present limitations in absorption kinetic studies, making ex vivo studies preferable since, despite the limitations, they can reproduce the kinetics region of the lung-region absorption. Moreover, ex vivo models allow for a complete study of most of cell types involved in the lung tissue (Cryan et al. 2007). Isolated perfused lung model, where the lung is outside the body, is commonly used as an ex vivo model. Even though the environment is artificial, the lung tissue keeps its architecture and functionality. The organ is maintained at a specific temperature and pressure, and it is pumped with perfusate while the drug is delivered by the tracheal port. In order to obtain the rate of absorption, the perfusate is sampled (Sakagami 2006; Cryan et al. 2007). Notwithstanding, in vivo methods enable the direct acquisition of pharmacokinetic data from lung tissue of live animals, usually small rodents, even though in this type of study, larger animals such as dogs, sheep, or monkeys are more appropriate (Sakagami 2006). Gamma scintigraphy is an in vivo non-invasive imaging technique that uses radiopharmaceuticals that localize in different organs and are visualized in a gamma camera to determine drug delivery precisely, by giving a measurement of the local bioavailability through the lung. The results obtained are an accurate percentage of the delivered dose and also a specific rate and extent of the drug's action (Newman and Wilding 1998; Zhang et al. 2011).

Clinical trials

Unlike other delivery systems, the current excipients approved by the FDA are very limited and not accepted worldwide. Such issue renders some difficulties in finding suitable polymeric- or lipid-based materials authorized for inhalation for this technique (Hu et al. 2013).

In 2007, Rogueda and Traini published a review paper about the delivery of nanoparticles through pulmonary route. At that time, publications were scarce, only one patent regarding DPI's and covering sizes below 500 nm was available, and there were no available products on the market for the delivery of nanoparticles using DPI (Rogueda and Traini 2007). The FDA approved the use of insulin DPIs (Exubera, Pfizer) in 2006, to treat patients with type 1 and type 2 diabetes, which was a major breakthrough for research in DPIs (Fineberg et al. 2005; Misra et al. 2011). However, one year later this product was removed from the market due to some uncertainties over patient compliance and long-term safety (Walle 2011; Misra et al. 2011). Notwithstanding, in June 2014 Technosphere® Technology from Mannkind Corporation announced FDA approval for their most recent treatment for diabetes AFREZZA®, an inhalation powder to improve glycemic control in adult patients with diabetes mellitus. However, the product presents some limitations: AFREZZA® must be used in combination with a long-acting insulin in patients with type 1 diabetes mellitus; and it is not recommended for the treatment of diabetic ketoacidosis nor for patients who smoke. Still, AFREZZA® is not currently distributed since there are some clinical trials lacking such as pharmacokinetics evaluation and pediatric patients safety and efficacy, potential risk of pulmonary malignancy, and also pharmacokinetic-pharmacodynamic euglycemic glucose-clamp clinical trials (Kling 2008).

Nolan and co-workers reported in 2011 an excipient-free formulation approach of making sodium

Table 6 Clinical trials using dry powder formulations for a wide variety of pulmonary diseases

Clinical trials	Condition/drug	Formulation/processing method	MMAD (μm)/FPF	Sponsor	Status
A twelve month long term safety study to evaluate the safety of albuterol in a dry powder inhaler with both repeated and as needed dosing	Asthma/ Albuterol	Levalbuterol L-tartare; surfactant (e.g., oleic acid)/Milling (McGlynn et al. 2007)	1.5–2.1/ 31.6–34.6 %	Teva Branded Pharmaceutical Products, R&D Inc.	Completed
Pharmacokinetic evaluation and tolerability of dry powder tobramycin by a novel device in patients with non cystic fibrosis bronchiectasis	Bronchiectasis/ Tobramycin	Tobramycin; sulfate; DSPC; Calcium chloride ($CaCl_2$)/spray drying (Challoner et al. 2012)	n.a.	University Medical Centre Groningen	Recruiting
A clinical trial to assess the safety of a measles vaccine (dry powder) administered by two different devices (PMV-001)	Measles Virus/ Live attenuated Edmonstron-Zagreb measles virus	Myo-inositol; Leucine/ supercritical fluid drying (ClinicalTrials.gov 2013)	n.a./ ~ 40%	Serum Institute of India Limited	Completed
To determine the relationship between baseline reversibility and the efficacy of indacaterol (REVERBREZ)	Chronic Obstructive Pulmonary Disease (COPD)/ Indacaterol	Indacaterol; glycopyrrolate; DSPC; trehalose; $CaCl_2$/spray drying (Weers et al. 2013)	2.3–2.8/ 57–69 %	Novartis Pharmaceuticals	Completed
Ciprofloxacin dry powder for inhalation in non-cystic fibrosis bronchiectasis (Non-CF BE) (RESPIRE 1)	Bronchiectas/ Ciprofloxacin	n.a. (ClinicalTrials.gov 2014)	n.a.	Bayer	Recruiting
Sensitivity of pharmacokinetics to differences in aerodynamic particle size distribution	Asthma/ Fluticasone Propionate	Fluticasone Propionate crystals/anti-solvent process (Ticehurst et al. 2014)	5.1–6.7/n.a.	University of Florida	Recruiting

n.a. not available

cromoglycate, an antiasthmatic, and anti-allergenic drug whose use in a DPI was firstly reported in 1998 into spherical-nanoporous microparticles via spray-drying technique (Laube et al. 1998; Nolan et al. 2011). The results obtained by this group showed improved aerodynamic properties comparing to the drug by itself demonstrating that porosity and sphericity of drug particles are preponderant features to enhance aerosol deposition (Nolan et al. 2011; Hu et al. 2013). Further optimization, regarding the aerodynamic properties, was achieved by Hu and collaborators by coupling continuous liquid solvent precipitation with immediate spray drying. Spherical agglomerates of pure drug nanoparticles were achieved with sizes more suitable for pulmonary delivery (4.46 ± 0.14 μm), thus increasing the FPF in ~ 50 % (Hu et al. 2013).

More recently, Nanotherapeutics Inc., challenged the National Institute of Allergy and Infectious Diseases (NIAD) to produce an inhaled version of the injectable antiviral drug Cidofovir for the treatment of smallpox and for non-invasive post-exposure prophylaxis. The development of inhaled Cidofovir could decrease the amount of people that are still vulnerable to smallpox due to their inability to be vaccinated. Several studies have already proved that this new respirable formulation of Cidofovir is very efficient against multiple pox models, when compared to injectable administration. In turn, the amount of powder retained in the pulmonary level is increased and, quite possibly avoids nephrotoxicity (so far caused by the injectable form). Amazingly, the use of gold nanoshells to deliver such drug, as already started in human models (Kaur et al. 2012). Moreover, a powder formulation of the measles

vaccine was prepared by a supercritical assisted process and went under clinical trial investigation (Tonnis et al. 2013). Table 6 summarizes the more-recent ongoing clinical trials on dry powder formulations for a wide range of pulmonary diseases. The listed dry powder formulations under clinical trials comprise crystalline and/or amorphous APIs blended in excipients of, for example, phospholipid, monosaccharides, disaccharides, polysaccharides, additols, and surfactants such as oleic acid and 1,2 distearoyl-sn-glycero-3-phosphocholine (DSCP). These formulations could be classified as particles with nanofeatures (as described in sub-Sect. 4.2).

Conclusion

The present review has highlighted the current status as well as the major benefits and limitations of the already existing and the under development formulations for "dry powder inhalers". Particle engineering is a fresh and *in vogue* area that combines chemical engineering, biopharmaceuticals, biology, formulation science, colloid and interface science, solid state physics, aerosol, and powder science and nanotechnology. Nanoparticulated systems have been showing a huge potential in the delivery of biological active substances. Recent progresses in aerosol formulations have led to the development of more efficient delivery systems able to produce small particle aerosols (nano-in-micro formulations) allowing higher powders deposition in the alveoli and deep lung region. The combination of nanotechnology and pulmonary delivery of aerosolizable drugs brings out new and exciting breakthroughs for the diagnosis and therapy of several types of lung and systemic diseases.

Particle engineering requires a deeper understanding of particle formation processes. A wide range of research experts from different but complementary areas, is already working in the design of safe and effective formulations to make these therapies safer and more effective. A detailed research in the possible combination of nano-in-micro dry powder formulations making use of spray drying and/or supercritical-assisted atomization is behind the scope of this review.

It is well known that the new class of engineered spray-dried particles and more specifically SAA formulations will definitely cause a shift in the production pipeline of pharmaceutical companies. Although this type of research appears to be promising in lung disease therapeutics, some difficulties still need to be overcome before the potential for wide public use may be appreciated. The fate and effect of the particles, specifically the nanoparticles release from the micro platforms, need to be fully understood before clinical trials may be performed. Safety and efficacy must be assured, and optimization processes must be guaranteed so that large scale manufacturing processes can be achieved, reducing costs and waste materials. Despite the plentiful advantages and applications of supercritical fluid technology, there is still room for improvement in the pharmaceutical industry. The influence of operating parameters on particle size and its morphology needs to be completely discerned. Potential efforts must be performed to defeat these challenges, as supercritical fluid technology has added a totally new dimension to the pharmaceutical research and formulation development. Moreover, successful design of engineered particles requires a thorough understanding and predictive modeling, so that the early development process can be completed in an acceptable time with a high likelihood of success. These are definitely the ultimate goals for the successful production and approval of dry powders produced through the use of sustainable strategies.

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References

Adami R, Osséo LS, Reverchon E (2009) Micronization of lysozyme by supercritical assisted atomization. Biotechnol Bioeng 104:1162–1170

Adami R, Liparoti S, Reverchon E (2011) A new supercritical assisted atomization configuration, for the micronization of thermolabile compounds. Chem Eng J 173:55–61

Akiyama Y, Mori T, Katayama Y, Niidome T (2009) The effects of PEG grafting level and injection dose on gold nanorod biodistribution in the tumor-bearing mice. J Control Release 139:81–84

Alderborn G, Elofsson U, Elversson J, Millqvist-fureby A (2003) Droplet and particle size relationship and shell thickness of inhalable lactose particles during spray drying. J Pharm Sci 92:900–910

Al-Qadi S, Grenha A, Carrión Recio D, Seijo B, Remuñán-López C (2012) Microencapsulated chitosan nanoparticles for pulmonary protein delivery: in vivo evaluation of

insulin-loaded formulations. J Control Release 157:383–390
Amidi M, Pellikaan HC, de Boer AH, Crommelin DJA, Hennink WE, Jiskoot W (2008) Preparation and physicochemical characterization of supercritically dried insulin-loaded microparticles for pulmonary delivery. Eur J Pharm Biopharm 68:191–200
Borm PJA, Kreyling W (2004) Toxicological hazards of inhaled nanoparticles—potential implications for drug delivery. J Nanosci Nanotechnol 4:521–531
Cabral RP (2013) Development of chitosan-based microparticles for pulmonary drug delivery. Dissertation, Univerdade Nova de Lisboa
Casimiro T, Barroso T, Figueiredo P, Costa E, Aguiar-Ricardo A (2011) Porous chitosan –drug formulations by scCO2-assisted atomization. In: Proceedings of 13th European meeting on supercritical fluids. http://www.isasf.net/fileadmin/files/Docs/DenHaag/HtmlDir/Papers/P68.pdf. Accessed 20 May 2014
Challoner P, Rodriguez C, Tarara T (2012) Tobramycin formulation for treatment of endobronchial infections European Patent 1765288 B1
Chattopadhyay P, Gupta RB (2001) Production of antibiotic nanoparticles using supercritical CO2 as antisolvent with enhanced mass transfer. Ind Eng Chem Res 40:3530–3539
Chattopadhyay P, Gupta RB (2002) Supercritical CO2 based production of magnetically responsive micro- and nanoparticles for drug targeting. Ind Eng Chem Res 41:6049–6058
Choi HS, Ashitate Y, Lee JH, Lee JH, Kim SH, Matsui A, Insin N, Bawendi MG, Semmler-Behnke M, Frangioni JV, Tsuda A (2010) Rapid translocation of nanoparticles from the lung airspaces to the body. Nat Biotechnol 28: 1300–1303
Chow AHL, Tong HHY, Chattopadhyay P, Shekunov BY (2007) Particle engineering for pulmonary drug delivery. Pharm Res 24:411–437
ClinicalTrials.gov (2013) A clinical trial to assess the safety of a measles vaccine (dry powder) administered by two different devices (PMV-001). http://clinicaltrials.gov/show/NCT01557699. Accessed 14 Oct 2014
ClinicalTrials.gov (2014) Ciprofloxacin dry powder for inhalation in non-cystic fibrosis bronchiectasis (Non-CF BE) (RESPIRE 1). http://clinicaltrials.gov/show/NCT01764841. Accessed 14 Oct 2014
Couzin-Frankel J (2013) Cancer immunotherapy. Science 342(20):1432–1433
Crowther Labiris NR, Holbrook AM, Chrystyn H et al (1999) Dry powder versus intravenous and nebulized gentamicin in cystic fibrosis and bronchiectasis. A pilot study. Am J Respir Crit Care Med 160:1711–1716
Cruz LJ, Tacken PJ, Fokkink R, Joosten B, Stuart MC, Albericio F, Torensma R, Figdor CG (2010) Targeted PLGA nano- but not microparticles specifically deliver antigen to human dendritic cells via DC-SIGN in vitro. J Control Release 144:118–126
Cryan S-A, Sivadas N, Garcia-Contreras L (2007) In vivo animal models for drug delivery across the lung mucosal barrier. Adv Drug Deliv Rev 59:1133–1151
Dreaden EC, Austin LA, Mackey MA, El-Sayed MA (2012) Size matters: gold nanoparticles in targeted cancer drug delivery. Ther Deliv 3:457–478
Dunbar CA, Concessio NM, Anthony J (1998) Evaluation of atomizer performance in production. Pharm Dev Technol 3:433–441
Duncan B, Kim C, Rotello VM (2010) Gold nanoparticle platforms as drug and biomacromolecule delivery systems. J Control Release 148:122–127
Elversson J, Millqvist-Fureby A (2005) Particle size and density in spray drying-effects of carbohydrate properties. J Pharm Sci 94:2049–2060
Fineberg SE, Kawabata T, Finco-Kent D, Liu C, Krasner A (2005) Antibody response to inhaled insulin in patients with type 1 or type 2 diabetes. An analysis of initial phase II and III inhaled insulin (Exubera) trials and a two-year extension trial. J Clin Endocrinol Metab 90:3287–3294
Gabrio BJ, Stein SW, Velasquez DJ (1999) A new method to evaluate plume characteristics of hydrofluoroalkane and chlorofluorocarbon metered dose inhalers. Int J Pharm 186:3–12
Geiser M, Quaile O, Wenk A, Wigge C, Eigeldinger-Berthou S, Hirn S, Schäffler M, Schleh C, Möller W, Mall MA, Kreyling WG (2013) Cellular uptake and localization of inhaled gold nanoparticles in lungs of mice with chronic obstructive pulmonary disease. Part Fibre Toxicol 10:19–29
Genina N, Räikkönen H, Heinämäki J, Veski P, Yliruusi J (2010) Nano-coating of β-galactosidase onto the surface of lactose by using an ultrasound-assisted technique. AAPS PharmSciTech 11:959–965
Gil M, Vicente J, Gaspar F (2010) Scale-up methodology for pharmaceutical spray drying. Chem Today 28:18–22
Girotra P, Singh SK, Nagpal K (2013) Supercritical fluid technology: a promising approach in pharmaceutical research. Pharm Dev Technol 18:22–38
Gómez-Gaete C, Fattal E, Silva L, Besnard M, Tsapis N (2008) Dexamethasone acetate encapsulation into Trojan particles. J Control Release 128:41–49
Grenha A, Remuñán-López C, Carvalho ELS, Seijo B (2008) Microspheres containing lipid/chitosan nanoparticles complexes for pulmonary delivery of therapeutic proteins. Eur J Pharm Biopharm 69:83–93
Hadinoto K, Zhu K, Tan RBH (2007) Drug release study of large hollow nanoparticulate aggregates carrier particles for pulmonary delivery. Int J Pharm 341:195–206
Hardy JG, Chadwick TS (2000) Sustained release drug delivery to the lungs: an option for the future. Clin Pharmacokinet 39:1–4
Hickey AJ, Mansour HM, Telko MJ, Xu Z, Smyth HD, Mulder T, McLean R, Langridge J, Papadopoulos D (2007) Physical characterization of component particles included in dry powder inhalers I. Strategy review and static characteristics. J Pharm Sci 96:1282–1301
Hoet PH, Brüske-Hohlfeld I, Salata OV (2004) Nanoparticles—known and unknown health risks. J Nanobiotechnol 2:12–27
Hu J, Dong Y, Pastorin G, Ng WK, Tan RBH (2013) Spherical agglomerates of pure drug nanoparticles for improved pulmonary delivery in dry powder inhalers. J Nanoparticle Res 15:1560–1572
Lai Sk, Wang Y-Y, Hanes J (2010) Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. Adv Drug Deliv Rev. 61:158–171

Kaur G, Narang RK, Rath G, Goyal AK (2012) Advances in pulmonary delivery of nanoparticles. Artif Cells Blood Substit Immobil Biotechnol 40:75–96

Kaye SR, Tol SP, Alpar HO (2009) Simultaneously manufactured nano-in-micro (SIMANIM) particles for dry-powder modified-release delivery of antibodies. J Pharm Sci 98:4055–4068

Kling J (2008) Inhaled insulin's last gasp? Nat Biotechnol 26:479–480

Klingler C, Müller BW, Steckel H (2009) Insulin-micro- and nanoparticles for pulmonary delivery. Int J Pharm 377:173–179

Koushik K, Dhanda DS, Cheruvu NPS, Kompella UB (2004) Pulmonary delivery of deslorelin: large-porous PLGA particles and HPbetaCD complexes. Pharm Res 21:1119–1126

Kurmi BD, Kayat J, Gajbhiye V, Tekade RK (2010) Micro- and nanocarrier-mediated lung targeting. Expert Opin Drug Deliv 7:781–794

Laube BL, Edwards AM, Dalby RN, Creticos PS, Norman PS (1998) Respiratory pathophysiologic responses: the efficacy of slow versus faster inhalation of cromolyn sodium in protecting against allergen challenge in patients with asthma. J Allergy Clin Immunol 101:475–483

Li H-Y, Birchall J (2006) Chitosan-modified dry powder formulations for pulmonary gene delivery. Pharm Res 23:941–950

Li Y-Z, Sun X, Gong T, Liu J, Zuo J, Zhang ZR (2010) Inhalable microparticles as carriers for pulmonary delivery of thymopentin-loaded solid lipid nanoparticles. Pharm Res 27:1977–1986

Linsenbühler M, Wirth K-E (2005) An innovative dry powder coating process in non-polar liquids producing tailor-made micro-particles. Powder Technol 158:3–20

Makadia HK, Siegel SJ (2011) Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel) 3:1377–1397

Malcolmson RJ, Embleton JK (1998) Dry powder formulations for pulmonary delivery. Pharm Sci Technolo Today 1:394–398

Mansour HM, Rhee Y-S, Wu X (2009) Nanomedicine in pulmonary delivery. Int J Nanomed 4:299–319

Martín A, Cocero MJ (2008) Micronization processes with supercritical fluids: fundamentals and mechanisms. Adv Drug Deliv Rev 60:339–350

Martín Á, Weidner E (2010) PGSS-drying: mechanisms and modeling. J Supercrit Fluids 55:271–281

Martín Á, Pham HM, Kilzer A, Kareth S, Weidner E (2010) Micronization of polyethylene glycol by PGSS (Particles from Gas Saturated Solutions)-drying of aqueous solutions. Chem Eng Process Process Intensif 49:1259–1266

McGlynn P, Bakale R, Sturge C (2007) Levalbuterol salt US Patent 7256310 B2 10

Millqvist-fureby A, Malmsten M (1999) Spray-drying of trypsin—surface characterisation and activity preservation. Int J Pharm 188:243–253

Misra A, Hickey AJ, Rossi C, Borchard G, Terada H, Makino K, Fourie PB, Colombo P (2011) Inhaled drug therapy for treatment of tuberculosis. Tuberculosis 91:71–81

Moghaddam PH, Ramezani V, Esfandi E, Vatanara A, Nabi-Meiobodi M, Darabi M, Gilani K, Najafabadi AR (2013) Development of a nano–micro carrier system for sustained pulmonary delivery of clarithromycin. Powder Technol 239:478–483

Mohajel N, Najafabadi AR, Azadmanesh K, Vatanara A, Moazeni E, Rahimi A, Gilani K (2012) Optimization of a spray drying process to prepare dry powder microparticles containing plasmid nanocomplex. Int J Pharm 423:577–585

Newman SP, Wilding IR (1998) Gamma scintigraphy: an in vivo technique for assessing the equivalence of inhaled products. Int J Pharm 170:1–9

Nolan LM, Li J, Tajber L, Corrigan OI, Healy AM (2011) Particle engineering of materials for oral inhalation by dry powder inhalers. II-Sodium cromoglycate. Int J Pharm 405:36–46

Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113:823–839

Odziomek M, Sosnowski TR, Gradoń L (2012) Conception, preparation and properties of functional carrier particles for pulmonary drug delivery. Int J Pharm 433:51–59

Oishi M, Tamura A, Nakamura T, Nagasaki Y (2009) A smart nanoprobe based on fluorescence-quenching PEGylated nanogels containing gold nanoparticles for monitoring the response to cancer therapy. Adv Funct Mater 19:827–834

Okamoto H, Nishida S, Todo H, Sakakur Y, Iida K, Danjo K (2003) Pulmonary gene delivery by chitosan-pDNA complex powder prepared by a supercritical carbon dioxide process. J Pharm Sci 92:371–380

Ozeki T, Beppu S, Mizoe T, Takashima Y, Yuasa H, Okada H (2006) Preparation of polymeric submicron particle-containing microparticles using a 4-fluid nozzle spray drier. Pharm Res 23:177–183

Packhaeuser CB, Lahnstein K, Sitterberg J, Schmehl T, Gessler T, Bakowsky U, Seeger W, Kissel T (2009) Stabilization of aerosolizable nano-carriers by freeze-drying. Pharm Res 26:129–138

Pasquali I, Bettini R, Giordano F (2006) Solid-state chemistry and particle engineering with supercritical fluids in pharmaceutics. Eur J Pharm Sci 27:299–310

Pilcer G, Amighi K (2010) Formulation strategy and use of excipients in pulmonary drug delivery. Int J Pharm 392:1–19

Powell MC, Kanarek MS (2006) Nanomaterial health effects—part 1: background and current knowledge. Wis Med J 105:16–20

Pulliam B, Sung JC, Edwards DA (2007) Design of nanoparticle-based dry powder pulmonary vaccines. Expert Opin Drug Deliv 4:651–663

Rehman M, Shekunov BY, York P, Lechuga-Ballesteros D, Miller DP, Tan T, Colthorpe P (2004) Optimisation of powders for pulmonary delivery using supercritical fluid technology. Eur J Pharm Sci 22:1–17

Restani RB, Conde J, Baptista PV, Cidade MT, Bragança AM, Morgado J, Correia I, Aguiar-Ricardo A, Bonifácio VDB (2014) Polyurea dendrimer for efficient cytosolic siRNA delivery. RSC Adv. doi:10.1039/C4RA0903G

Reverchon E (1999) Supercritical antisolvent precipitation of micro- and nano-particles. J Supercrit Fluids 15:1–21

Reverchon E (2002) Supercritical-assisted atomization to produce micro- and/or nanoparticles of controlled size and distribution. Ind Eng Chem Res 41:2405–2411

Reverchon E (2007) Process for the production of micro and/or nano particles US Patent 7276190 B2. 10

Reverchon E, Adami R (2006) Nanomaterials and supercritical fluids. J Supercrit Fluids 37:1–22

Reverchon E, Adami R, Caputo G (2006) Supercritical assisted atomization: Performance comparison between laboratory and pilot scale. J Supercrit Fluids 37:298–306

Rogueda PG, Traini D (2007) The nanoscale in pulmonary delivery. Part 2: formulation platforms. Expert Opin Drug Deliv 6:607–620

Rowe RC, Sheskey PJ, Quinn ME (2009) Handbook of pharmaceutical excipients. Pharmaceutical Press, London

Sadhukha T, Wiedmann TS, Panyam J (2013) Inhalable magnetic nanoparticles for targeted hyperthermia in lung cancer therapy. Biomaterials 34:5163–5171

Sakagami M (2006) In vivo, in vitro and ex vivo models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. Adv Drug Deliv Rev 58:60–1030

Sanli D, Bozbag SE, Erkey C (2011) Synthesis of nanostructured materials using supercritical CO2: Part I. Physical transformations. J Mater Sci 47:2995–3025

Sinsuebpol C, Chatchawalsaisin J, Kulvanich P (2013) Preparation and in vivo absorption evaluation of spray dried powders containing salmon calcitonin loaded chitosan nanoparticles for pulmonary delivery. Drug Des Devel Ther 7:861–873

Sivadas N, O'Rourke D, Tobin A, Buckley V, Ramtoola Z, Kelly JG, Hickey AJ, Cryan SA (2008) A comparative study of a range of polymeric microspheres as potential carriers for the inhalation of proteins. Int J Pharm 358:159–167

Son Y-J, Worth Longest P, Hindle M (2013) Aerosolization characteristics of dry powder inhaler formulations for the excipient enhanced growth (EEG) application: effect of spray drying process conditions on aerosol performance. Int J Pharm 443:137–145

Stegemann S, Kopp S, Borchard G, Shah VP, Senel S, Dubey R, Urbanetz N, Cittero M, Schoubben A, Hippchen C, Cade D, Fuglsang A, Morais J, Borgström L, Farshi F, Seyfang KH, Hermann R, van de Putte A, Klebovich I, Hincal A (2013) Developing and advancing dry powder inhalation towards enhanced therapeutics. Eur J Pharm Sci 48:181–194

Stephenson GA, Forbes RA, Reutzel-Edens SM (2001) Characterization of the solid state: quantitative issues. Adv Drug Deliv Rev 48:67–90

Storey RA, Ingvar Y (2011) Solid state characterization of pharmaceuticals. Wiley, Hoboken

Sung JC, Padilla DJ, Garcia-Contreras L, Verberkmoes JL, Durbin D, Peloquin CA, Elbert KJ, Hickey AJ, Edwards DA (2009) Formulation and pharmacokinetics of self-assembled rifampicin nanoparticle systems for pulmonary delivery. Pharm Res 26:55–1847

Telko MJ, Dsc AJH (2005) Dry powder inhaler formulation. Respir Care 50:1209–1227

Tewa-Tagne P, Briançon S, Fessi H (2006) Spray-dried microparticles containing polymeric nanocapsules: formulation aspects, liquid phase interactions and particles characteristics. Int J Pharm 325:63–74

Ticehurst M, Marziano I, Kougoulos E (2014) Process for the preparation of fluticasone propionate form 1 US Patent 0141247 A1 14

Tolman JA, Williams RO (2010) Advances in the pulmonary delivery of poorly water-soluble drugs: influence of solubilization on pharmacokinetic properties. Drug Dev Ind Pharm 36:1–30

Tonnis WF, Lexmond AJ, Frijlink HW, de Boer AH, Hinrichs WLJ (2013) Devices and formulations for pulmonary vaccination. Expert Opin Drug Deliv 10:1383–1397

Tsapis N, Bennett D, Jackson B, Weitz DA, Edwards DA (2002) Trojan particles: large porous carriers of nanoparticles for drug delivery. Proc Natl Acad Sci USA 99:12001–12005

Türk M (1999) Formation of small organic particles by RESS : experimental and theoretical investigations. J Supercrit Fluids 15:79–89

Ungaro F, D'Angelo I, Miro A, La Rotonda MI, Quaglia F (2012) Engineered PLGA nano-and micro-carriers for pulmonary delivery: challenges and promises. J Pharm Pharmacol 64:1217–1235

Van Der Walle C (2011) Peptide and protein delivery. Elsevier, Oxford

Vehring R (2008) Pharmaceutical particle engineering via spray drying. Pharm Res 25:999–1022

Wanakule P, Liu GW, Fleury AT, Roy K (2012) Nano-inside-micro: disease-responsive microgels with encapsulated nanoparticles for intracellular drug delivery to the deep lung. J Control Release 162:429–437

Weers J, Rao N, Huang D, Miller D, Tarara T (2013) Dry powder formulation of particles that contain two or more active ingridients for treating obstructive or inflammatory airways diseases US Patent 0319411 A1 13

Yang W, Peters JI, Williams RO (2008) Inhaled nanoparticles–a current review. Int J Pharm 356:239–247

Yang Y, Bajaj N, Xu P, Ohn K, Tsifansky MD, Yeo Y (2009) Development of highly porous large PLGA microparticles for pulmonary drug delivery. Biomaterials 30:1947–1953

Yang L, Luo J, Shi S, Zhang Q, Sun X, Zhang Z, Gong T (2013) Development of a pulmonary peptide delivery system using porous nanoparticle-aggregate particles for systemic application. Int J Pharm 451:104–111

Yeo S-D, Kiran E (2005) Formation of polymer particles with supercritical fluids: a review. J Supercrit Fluids 34:287–308

Zhang J, Wu L, Chan H-K, Watanabe W (2011) Formation, characterization, and fate of inhaled drug nanoparticles. Adv Drug Deliv Rev 63:441–455