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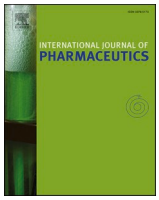

Review

A Review on Micro and Nanoengineering in Powder-Based Pulmonary Drug Delivery

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ABSTRACT

Pulmonary delivery of drugs has emerged as a promising approach for the treatment of both lung and systemic diseases. Compared to other drug delivery routes, inhalation offers numerous advantages including high targeting, fewer side effects, and a huge surface area for drug absorption. However, the deposition of drugs in the lungs can be limited by lung defence mechanisms such as mucociliary and macrophages' clearance. Among the delivery devices, dry powder inhalers represent the optimal choice due to their stability, ease of use, and absence of propellants. In the last decades, several bottom-up techniques have emerged over traditional milling to produce inhalable powders. Among these techniques, the most employed ones are spray drying, supercritical fluid technology, spray freeze-drying, and thin film freezing. Inhalable dry powders can be constituted by micronized drugs attached to a coarse carrier (e.g., lactose) or drugs embedded into a micro- or nanoparticle. Particulate-based formulations are commonly composed of polymeric micro- and nanoparticles, liposomes, solid lipid nanoparticles, dendrimers, nanocrystals, extracellular vesicles, and inorganic nanoparticles. Moreover, engineered formulations including large porous particles, swellable microparticles, nano-in-microparticles, and effervescent nanoparticles have been developed. Particle engineering has also a crucial role in tuning the physical–chemical properties of both carrier-based and carrier-free inhalable powders. This approach can increase powder flowability, deposition, and targeting by customising particle surface features.

1. Introduction

Inhalation therapy has been practised since ancient times. Its use is reported in an Egyptian papyrus, dating around 1554 BCE, describing the treatment of breathless patients with black henbane vapours (Sanders, 2007; Stein and Thiel, 2017). It is known that plants such as Ephedra sinica and Datura stramonium were traditionally burnt and inhaled to treat asthma in China and India, respectively (Andrade et al., 2013). Furthermore, smoking opium was widespread in China around 1100 BCE for the same purpose (Stein and Thiel, 2017), and the therapeutic inhalation of herbal vapours was also common among the population of South and Central America and Greece (Sanders, 2007; Stein and Thiel, 2017). Important advances in inhalation therapy were made from 1760 to 1955, thanks to the development of the first delivery systems. Afterwards, between 1956 and 1986, a major improvement in aerosolization techniques and delivery devices paved the way for the modern administration of drugs through nebulisers, pressurised metered dose inhalers (pMDIs), soft mist inhalers (SMIs), and dry powder inhalers (DPIs) (Stein and Thiel, 2017).

The pulmonary route has gained much interest during the last decades among the various drug administration methods. This technique mainly delivers bronchodilators and corticosteroids to lungs affected by asthma or chronic obstructive pulmonary disease (COPD) (Newman, 2018; Ramadan et al., 2019; Shakshuki and Agu, 2017). The treatment of less common respiratory diseases such as tuberculosis (TB) (Chae et al., 2021; Miranda et al., 2018; Nainwal et al., 2022), cystic fibrosis (CF) (d'Angelo et al., 2014; Garbuzenko et al., 2019; Savla and Minko, 2013), pulmonary arterial hypertension (PAH) (Plaunt et al., 2021; Teymouri Rad et al., 2019), and lung cancer (Abdelaziz et al., 2018) can also be a target (Newman, 2017). However, although inhalable chemotherapy allows for improved efficacy and fewer side effects compared to systemic chemotherapy, its use in the clinical setting is not widespread (Abdelaziz et al., 2018). Other than being used to treat respiratory diseases, pulmonary drug delivery can be exploited to promote systemic absorption of drugs in the alveolar region of the lungs

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Nomenclature

Abbreviations

ADiC	Aerosol Dynamics in Containments
BDP	Beclomethasone dipropionate
CF	Cystic Fibrosis
CLF	Coarse Lactose Fines
COPD	Chronic Obstructive Pulmonary Disease
CS	Chitosan
DPCC	Dipalmitoylphosphatidylcholine
DPI	Dry Powder Inhaler
EC	Ethyl Cellulose
EV	Extracellular vesicle
FDA	Food and Drug Administration
FDKP	Fumaryl diketopiperazine
FLF	Fine Lactose Fines
FPF	Fine Particle Fraction
GSD	Geometric Standard Deviation
GUV	Giant Unilamellar Vesicle
HA	Hyaluronic acid
HPβCD	Hydroxypropyl-β-cyclodextrin
IDEAL	Inhalation, Deposition, and Exhalation of Aerosols in the Lungs
iSPERSE	inhaled Small Particles Easily Respirable and Emittable
L-leu	L-Leucine
LPP	Large Porous Particle
LUV	Large Unilamellar Vesicle
MgSt	Magnesium stearate
miRNA	microRNA
MISEV	Minimal Information for Studies of Extracellular Vesicles
MLV	Multilamellar Vesicles
MMAD	Mass Median Aerodynamic Diameter
MNP	Magnetic Nanoparticle
MP	Microparticle
MSN	Mesoporous Silica Nanoparticles
MV	Microvesicle
NaCl	Sodium chloride
NEP	Nanocrystals-Embedded Microparticle
NiM	Nano-in-Microparticle
NP	Nanoparticle
PAH	Pulmonary Arterial Hypertension
PAMAM	Polyamidoamine
pDNA	plasmid DNA
PEG	Polyethylene glycol
PEI	Polyethylenimine
PLGA	Poly(lactic-co-glycolide acid)
pMDI	pressurized Metered Dose Inhaler
PNAP	Porous Nanoparticle-Aggregate Particle
PRINT	Particle Replication In Nonwetting Templates
PS	Polystyrene
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
SAS	Supercritical Anti-Solvent
$scCO_2$	Supercritical Carbon Dioxide
SCF	Supercritical Fluid Technology
SD	Spray Drying
SF	Spray Freezing
SFD	Spray Freeze-Drying
siRNA	small-interfering RNA
SLN	Solid Lipid Nanoparticle
SMI	Soft Mist Inhaler
SS	Salbutamol Sulphate
SUV	Small Unilamellar Vesicle
TB	Tuberculosis
TFF	Thin Film Freezing

List of symbols

C_s	Cunningham slip correction factor
d_a	Particle aerodynamic diameter
ρ_a	Air density
ρ_s	Unit density
A	Particle area
d	Particle diameter
D	Diffusion coefficient
ER	Elongation ratio
g	Gravitational constant
k	Boltzmann constant
L	Length of a particle
p	Particle perimeter
R	Airway radius
RO	Roundness
Stk	Stokes' number
T	Absolute temperature
u	Particle linear velocity
w	Width of a particle
λ	Dynamic shape factor
μ	Dynamic viscosity of air
ν	Terminal settling velocity
ρ	Particle density

(Labiris and Dolovich, 2003a). Although small molecules are the most suitable to be inhaled to achieve a systemic effect due to their rapid absorption, peptide and protein-based drugs, such as insulin, can also be employed. In fact, after the release of the first inhalable insulin product Exubera® (Pfizer, NY, USA) in 2006, Food and Drug Administration (FDA) approved in 2015 another insulin product for inhalation (Afrezza®, Mannkind, CA, USA) (Fleming et al., 2015) based on the innovative Technosphere® technology (MannKind, CA, USA) (Jain et al., 2020; Newman, 2017). Higher bioavailability and reduced risk of hypoglycaemia can be obtained in patients with type 1 diabetes treated with inhalable insulin, according to recent studies (Seaquist et al., 2020). Inhalation is widely spreading as a route of administration in the vaccination field, showing promising outcomes (Masjedi et al., 2022). For example, inhalable vaccines against influenza, measles, and papillomavirus type-16 are under clinical trial (Heida et al., 2022), and the first inhaled Covid vaccine Convidecia Air (CanSino Biologics Inc, Tianjin, China) has been recently approved in China.

The interest in pulmonary delivery is growing, leading to the repurposing of several drugs previously administered only by the oral or parenteral route (Newman, 2018). This drug delivery method is of considerable interest since inhalation therapy enables delivery to different districts of the respiratory system for local and systemic treatments. The high targeting efficiency of pulmonary delivery lowers the doses of drug required to achieve a rapid clinical response, thus minimising systemic side effects, and reducing drug costs (Newman, 2018, 2017). Moreover, barriers such as first-pass metabolism and enzymatic degradation are bypassed, hence promoting higher therapeutic effectiveness (Newman, 2018). Lastly, whether applied for the treatment of systemic diseases, pulmonary delivery can be preferred to other administration routes since it is needle-free and it offers a huge alveolar surface area (>100 m^2) for drug absorption (Labiris and Dolovich, 2003a; Newman, 2018, 2017).

Despite these advantages, pulmonary drug delivery is challenging since, to achieve a therapeutic effect, inhalation of drugs must overcome

mechanical, chemical, and immunological barriers (Newman, 2017). These obstacles are mainly related to physiologic lung defence mechanisms, which aim to prevent particles from entering the respiratory system. For example, the largest particles are subjected to inertial impaction in nasal and oropharyngeal airways, while mucociliary clearance retains particles of size greater than 6 μm in the upper airways (El-Sherbiny et al., 2015). On the other hand, smaller particles can undergo alveolar macrophage clearance depending on particle size, shape, and surface chemistry (Murgia et al., 2014). In the second place, the presence of chemicals, e.g., proteolytic enzymes including antitrypsin, protease, and trypsin (He et al., 2022), can constitute a chemical barrier that may reduce drug bioavailability. Since other factors limiting pulmonary drug delivery efficiency can be the incorrect use of the inhaler device and poor adherence to the treatment regimen (Newman, 2017), patients need to be accurately instructed on the use of inhalers (Labiris and Dolovich, 2003b).

Nebulisers, pMDIs, and SMIs are based on liquid formulations, while DPIs are based on dry respirable particles inserted into the device in the shape of a static powder bed. The aerosolization process of dry powders in DPIs starts with the detachment of particles from the bed caused by the patient's inhalation, followed by fluidization and entrainment in the airstream. Resuspension and deposition are the last phases of this process (Peng et al., 2016). DPIs have received much attention owing to their unique advantages (de Boer et al., 2017), making them particularly useful for delivering poorly water-soluble and protein-based drugs (Muralidharan et al., 2015). DPIs are breath-actuated, transportable, compact, and easier to use than pMDIs since no inhalation-actuation coordination is required (Labiris and Dolovich, 2003b; Pramanik et al., 2021). The use of dry formulation of drugs offers more long-term stability and sterility than the aqueous one (Sung et al., 2007). Furthermore, the use of propellants is avoided, as well as cold chain storage (Hoppentocht et al., 2014; Muralidharan et al., 2015). However, particles in solid formulations also demonstrate some drawbacks since they are exposed to humidity, which may cause their aggregation, and they need strong and fast inhalations to detach particles from the static powder bed, making them unsuitable for children (Labiris and Dolovich, 2003b; Peng et al., 2016; Pramanik et al., 2021). Three types of DPIs are currently available on the market: unit dose, multidose, and multi-unit dose ones (Muralidharan et al., 2015), which are also classified in passive and active inhalers, depending on whether aerosolization is influenced or not by the patient's inspiratory flow (Duong et al., 2021). Unit-dose DPIs, such as Breezhaler® (Novartis, Basel, Switzerland) and HandiHaler® (Boehringer, Ingelheim am Rhein, Germany) can carry a single-drug-dose prepackaged in a capsule. Instead, Diskhaler® (GSK, London, UK), Diskus® (GSK, London, UK), Gyrohaler® (Vectura, Chippenham, UK) are examples of multi-unit-dose DPIs, which contain multiple single-dose capsules in the same device. At the same time, multidose devices are filled with a powder bed and can autonomously deliver the right amount of drug through a metering mechanism (Muralidharan et al., 2015). Turbuhaler® (AstraZeneca, Cambridge, UK), Easyhaler® (Orion, Espoo, Finland), Nexthaler® (Chiesi, Parma, Italy), Twisthaler® (MSD, Readington, USA), and Novolizer® (Meda, Milano, Italy) are examples of marketed multidose DPIs (Hoppentocht et al., 2014).

2. Deposition of particles in the lung

Respirable particles range between 1 nm and 10 μm and can deposit in human lungs by sticking to the wet surface of lung airways (Holger Schulz et al., 2000; Madl and Majid, 2011). The deposition should occur in the appropriate lung site to achieve the desired effect, depending on the requested therapeutic action, i.e., local or systemic (Labiris and Dolovich, 2003a). Particle deposition in the respiratory system is related both to the physiology of patients and the properties of the inhaled particles, such as size, shape, charge, density, and hygroscopicity (Muralidharan et al., 2015; Williams et al., 2011). The mechanisms involved in pulmonary deposition are inertial impaction, sedimentation, diffusion, interception, and electrostatic precipitation (Fig. 1). The former three mainly depend on particle size, while the latter two depend on particle shape and charge, respectively. Impaction occurs in the upper airways when particles do not follow the airflow curvature but keep their original trajectory, impacting the lung walls. This deposition mechanism is driven by inertia and is enhanced by the increase in the flow rate and particle size (Madl and Majid, 2011). The probability of inertial deposition is proportional to the Stokes' number (Stk) (Williams et al., 2011), which, for a spheric particle moving into a medium, is defined as follows:

$$\mathrm{Stk} = \frac{\rho d^2 u}{18 \mu R} \tag{1}$$

where ρ is the density of the particle, d is particle diameter, u is the particle linear velocity, μ is the dynamic viscosity of the medium (air), and R is the airway radius (Schulz et al., 2000; Williams et al., 2011).

Sedimentation is the deposition of inhaled particles on the surface of lower airways due to gravity (Madl and Majid, 2011) and the terminal settling velocity ν is:

$$\nu = \frac{(\rho - \rho_a) d^2 g C_s}{18 \mu} \tag{2}$$

where ρ_a is the air density and g is the gravitational constant. C_s is the Cunningham slip correction factor, which should be considered for particles smaller than 10 μm (Schulz et al., 2000; Williams et al., 2011). While sedimentation prevails for large particles and high flow rates, diffusion predominates for small particles and low flow rates, i.e., in the alveolar region (Madl and Majid, 2011). This mechanism is based on the Brownian motion, characterised by the diffusion coefficient D:

$$D = \frac{kTC_s}{3\pi\mu d} \tag{3}$$

where k is the Boltzmann constant and T is the absolute temperature (Schulz et al., 2000).

Interception and electrostatic precipitation are less relevant than the other deposition mechanisms. Interception is significant only for fibrous particles and is caused by the contact between the airways surface and particle edges (Schulz et al., 2000). On the other hand, electrostatic precipitation requires surface-charged particles, whose deposition is influenced by space-charge and image-charge forces. The former represents the repulsion among particles, while the latter refers to the attractive forces between particles and the lung wall and is relevant in the alveolar region for low-density particles (Kaialy, 2016a).

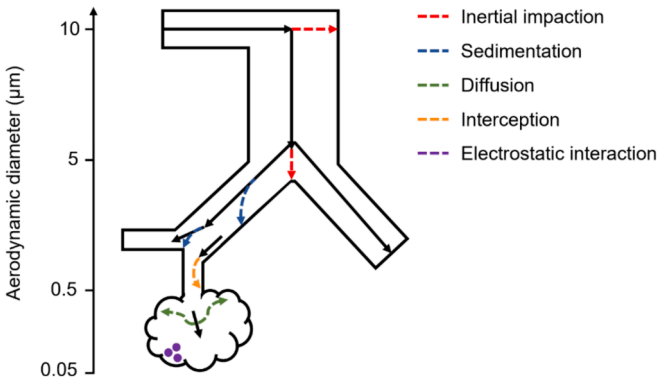

Fig. 1. Mechanisms of particle deposition in human airways and influence of the d_a on the deposition pattern.

2.1. Influence of particle size

Several studies, including Computational Fluid Dynamics simulations, have been performed to understand the relationship between particle size and particulate deposition in human airways (Fontana et al., 2005; Ou et al., 2020; Piglione et al., 2012). Besides particle geometric size, a particle's most relevant dimensional feature is its aerodynamic diameter, d_a, which is the diameter of a spherical particle with the same settling velocity and density of 1 g/cm^3:

$$d_a = d\sqrt{\frac{\rho}{\lambda\rho_s}} \quad (4)$$

where ρ is the density of the particle, ρ_s is the unit density (1 g/cm^3), d is the geometrical particle diameter, and λ is the dynamic shape factor of the particle (Hassan and Lau, 2009; Sung et al., 2007), which varies from 1.1 mm to 1.75 mm for non-spherical particles (Jain et al., 2020). An aerosol is defined by the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). MMAD is the particle size with respect to which half of the aerosol is smaller, and the other half is larger, while GSD represents the variability in particle diameters with respect to the MMAD (Jain et al., 2020; Labiris and Dolovich, 2003a). A value of GSD smaller than 1.2 indicates monodisperse aerosols, while polydisperse aerosols refer to GSD exceeding 1.2 (Schulz et al., 2000).

According to their geometric size, particles are classified as ultrafine (<0.1 μm), fine (0.1 μm −2 μm) and coarse (>2 μm) (El-Sherbiny et al., 2015). Once inhaled, each category has a different fate and is subjected to a precise deposition mechanism depending on its aerodynamic properties (Fig. 1). Particles of MMAD larger than 5 μm mainly impact on walls of the tracheobronchial region, where they undergo mucociliary clearance (Praphawatvet et al., 2020), and if their MMAD exceeds 10 μm they deposit in the oral cavity (Jain et al., 2020). Particles of aerodynamic size between 1 μm and 5 μm sediment in the smaller airways of bronchioles and alveoli, where they are exposed to the action of macrophages (El-Sherbiny et al., 2015; Jain et al., 2020; Praphawatvet et al., 2020). Particles with MMAD < 0.5 μm are driven by Brownian diffusion in the alveolar region (Praphawatvet et al., 2020) even though they are mostly exhaled for their low inertia (El-Sherbiny et al., 2015; Praphawatvet et al., 2020).

Particle size also affects the strength of cohesive forces among particles, which is significant when their d_a is smaller than 1 μm and negligible if it exceeds 5 μm. Since the cohesiveness should not be too high nor too low to ensure aerosolization, particles should have a d_a between 1 μm and 5 μm to achieve a good deposition into deep lungs (Chaurasiya and Zhao, 2021). This optimal size range avoids both inertial impaction and exhalation, ensuring at the same time adequate cohesion between particles (Praphawatvet et al., 2020). The level of drug deposition in the lung is associated with a high number of fine particles in the aerosol, i.e., the fine particle fraction (FPF) (Pramanik et al., 2021), which depends on the formulation and inhalation device (Peng et al., 2016). For example, in DPIs the FPF and the lung deposition depend not only on the d_a but also on the inspiratory flow rate as deeply discussed by Weers, 2022.

2.2. Influence of particle shape

The interception of particles in narrow airways is strictly related to particle shape (Jain et al., 2020), which influences the aerosolization properties of particles, including drag forces and terminal velocities (Peng et al., 2016). For instance, particles with irregular shapes are generally less prone to aggregation than homogeneous ones (Chaurasiya and Zhao, 2021). Moreover, slightly elongated particles, such as needle-shaped ones, generally exhibit good deep lung deposition but the dispersion of such particles in DPIs is a tricky issue (Jain et al., 2020). Hassan *et al.* found out that spherical and pollen shapes can improve flowability, aerosolization, and deposition of particles in lungs (Hassan and Lau, 2009) since spherical and pollen particles present higher FPF than needle-shaped ones, even though they have bigger d_a (Chaurasiya and Zhao, 2021). In addition, particle shape is essential in particle clearance by alveolar macrophages. Elongated particles are less internalised than spherical ones; for instance, rod-shaped particles can escape immune system recognition, while filamented and worm-like ones are less affected by macrophages' phagocytosis (El-Sherbiny et al., 2015; Patel et al., 2015; Yoo and Mitragotri, 2010).

It is important to notice that the shape of both the drug and the carrier particles should be considered when a micronized drug is physically attached to a coarse carrier (Peng et al., 2016).

2.3. Influence of particle charge

The deposition of inhaled drugs in the lungs by electrostatic interaction depends on particle charge (Chaurasiya and Zhao, 2021), as confirmed by several studies (Koolpiruck et al., 2004; Koullapis et al., 2016; Saini et al., 2004). Generally, charged particles show better deposition than neutral ones (Praphawatvet et al., 2020).

The surface charge of particles affects the interaction between particles and mucin, i.e., the negatively charged glycoprotein composing airway mucus (Praphawatvet et al., 2020; Scherließ et al., 2022). Therefore, mucociliary clearance can be escaped through surface charge regulation. For example, mucus penetration is encouraged for hydrophilic particles characterised by neutral or moderate negative charge, while cationic and hydrophobic particles preferentially undergo mucoadhesion (Murgia et al., 2014). Optimistically, a balance between mucus penetration and mucoadhesion should be obtained to ensure both deep-lung deposition and a proper residence time of drugs in the lung (Scherließ et al., 2022).

2.4. Influence of particle hygroscopicity

Since the lung is an extremely humid environment (relative humidity 99.5 %), particle hygroscopicity should be considered. Whether they are hypertonic or hypotonic, particles may swell or shrink, respectively, maybe altering the deposition site and pattern (Labiris and Dolovich, 2003a). Therefore, mathematical models have been developed to predict the influence of particle hygroscopicity on lung deposition (Asgharian, 2004; Winkler-Heil et al., 2017). Winkler-Heil et al. (2017) combined the aerosol model ADiC (Aerosol Dynamics in Containments) and the deposition model IDEAL (Inhalation, Deposition, and Exhalation of Aerosols in the Lungs) to calculate relative humidity, hygroscopic growth, and deposition of inhaled sodium chloride (NaCl) particles in human lungs. The results of this study were compared with other existing models and experimental data obtained by inhalation experiments with NaCl in human volunteers. Experimental and simulation analyses stated that particle size change in respiratory airways mainly depended on initial particle diameter, temperature, and relative humidity in the lungs (Asgharian, 2004). The addition of excipients, e.g., L-leucine (L-leu) and magnesium stearate (MgSt), can inhibit particles from moisture-induced size changes (Zhou and Morton, 2012; Zillen et al., 2021). On the other hand, the humidity-sensibility of particles can also be exploited to escape alveolar phagocytosis due to the particles' size increase, making them no longer attractive for lung macrophages (Liang et al., 2015).

3. Particulate-based formulations for pulmonary drug delivery

To be inhaled, drugs must be formulated as respirable powders with adequate size and aerodynamic behaviour, such as microparticles (MPs) or nanoparticles (NPs) (Miranda et al., 2018). In DPIs formulations, these particles can be either loose or blended to a non-respirable coarse carrier (Liang et al., 2015; Muralidharan et al., 2015). In the first case, dry particles with desired size are obtained from a solution of drug and excipients (Fig. 2a), while in the second approach, micronized drug and

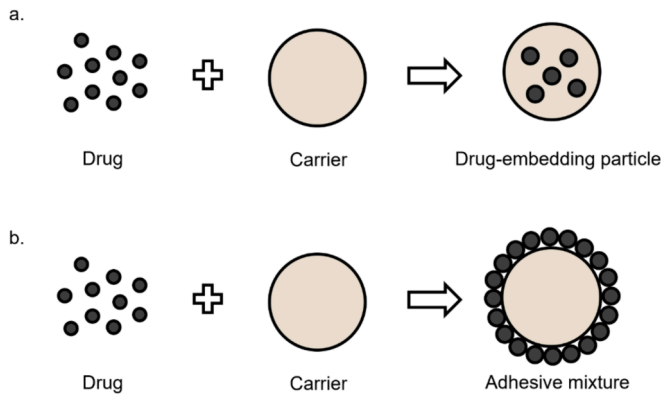

Fig. 2. Comparison of (a) dry particles obtained by a solution of drug and carrier and (b) adhesive mixture resulting from blending drug and carrier by means of their interactions.

coarse carrier are blended through their interactions (Fig. 2b) (Peng et al., 2016). A third strategy involves the formation of spherical soft agglomerates of micronized drugs and excipients, which dissociate in individual MPs after inhalation (Malcolmson and Embleton, 1998; Scherließ et al., 2022). Even though carrier-based interactive blends (i.e., adhesive mixtures) represent the traditional delivery system, the development of formulations devoid of coarse carriers has been deeply analysed since they eliminate technical and safety issues related to the presence of the large carrier (e.g., lactose) (Healy et al., 2014). Examples of carrier-free formulations are large porous particles (LPPs), Pulmosol™, Pulmosphere®, and Technosphere® technology, which was developed to deliver insulin (Afrezza®) and exploits the ability of the excipient fumaryl diketopiperazine (FDKP) to self-assemble in MPs (Healy et al., 2014). The selection of the optimal carrier and formulation is crucial to ensure the efficacy of the therapy (Muralidharan et al., 2015). A detailed overview of natural and bioinspired excipients for DPI formulations was given by Zillen et al. (2021). Aminoacids, such as L-leu and trileucine are mainly employed to reduce particle cohesiveness, while sugars are used as coarse carriers in adhesive mixtures, as microparticulate matrix for NPs, and as stabilisers in carrier-free formulations (Zillen et al., 2021). Lipids and phospholipids are also employed as excipients for DPI formulations (Zillen et al., 2021); for instance, MgSt, i.e., a synthetic salt with a lipid part, is used as a coating agent for both large carriers and micronized drugs (Kumar et al., 2022; Zillen et al., 2021). Biodegradable polymers can also be added to DPI formulations to promote sustained release (Zillen et al., 2021).

3.1. Carrier-based adhesive mixtures

MPs of a size compatible with deep-lung deposition (1–5 μm) are characterised by significant surface free energy and surface electric forces, which cause their aggregation and make the powder cohesive, with reduced flowability and poor dispersing properties. To solve this issue, micronized drugs are usually blended with coarse carrier particles (30–200 μm) (Labiris and Dolovich, 2003b; Peng et al., 2016). Different formulations are employed, depending on the content of the drug; in particular, adhesive mixture and soft spherical agglomerates are used for low doses of drug (μg), while soft pellets are preferable for high dose formulations (mg) such as antibiotics (Hoppentocht et al., 2014).

Adhesive mixtures are constituted by drug MPs adhered to the surface of a carrier (Rahimpour et al., 2014), whose delivery to the target site of the lung takes place in four steps. Powders are initially fluidized and dispersed by air insufflation in the DPI; then, the complex drug carrier is transported to the mouth. Once inhaled, the drug separates from the carrier to reach the lung, while the carrier is deposited in the oropharynx, and is swallowed (Zhang et al., 2019). The formation of the blend is influenced by various parameters, including type of mixer, operation time, and blending speed (Kaialy, 2016b). Also, the interactions between the drug and the carrier must be tuned to guarantee their adhesion during the blending process, allowing, at the same time, particle separation after inhalation (Malcolmson and Embleton, 1998). Van der Waals, electrostatic, interlocking, and capillary forces control these processes. In addition, the physical properties of the carrier, such as size, shape, and roughness, highly affect drug-carrier interactions and, consequently, the release of therapeutic agents to the lung (Peng et al., 2016). Therefore, the choice of the carrier is an essential step during the DPI formulation process (Rahimpour et al., 2014).

The most common carrier in pulmonary delivery is α-lactose monohydrate (Kaialy et al., 2012; Kaialy and Nokhodchi, 2012; Rahimpour and Hamishehkar, 2012) since it is stable, biodegradable, biocompatible, safe, and cost-effective (Momin et al., 2011). Although FDA has approved the use of lactose as a carrier for DPI formulations, lactose is not suitable for lactose-intolerant patients and for the delivery of some drugs (e.g., formoterol, peptide, and protein drugs) since it is a reducing sugar. Accordingly, several studies have investigated alternative carriers such as mannitol, trehalose, erythritol, sorbitol, xylitol, and maltitol (Rahimpour et al., 2014; Steckel and Bolzen, 2004). Among them, mannitol seems to be the best alternative since it is animal-free, extraordinarily stable (Hertel et al., 2020a), and, being a non-reducing sugar; it can be employed for peptide and protein-based drugs (Hertel et al., 2020b). For instance, Bronchitol®, a mannitol-based inhalation product, has already been approved by the FDA for treating CF and chronic bronchitis (Rahimpour et al., 2014). A recent study highlighted the similarity and superiority of mannitol, compared to lactose, as a carrier for the administration of the bronchodilator salbutamol sulphate (SS) and the corticosteroid budesonide, respectively (Hertel et al., 2020a). In addition, mannitol exhibited better FPF than glucose, sorbitol, and maltitol in the dry powder formulation of budesonide (Momin et al., 2011).

3.2. Microparticles

3.2.1. Polymeric microparticles

Drug-loaded MPs are commonly polymer-based and are usually incorporated into a sugar-based matrix (Zillen et al., 2021). The most used polymers are chitosan (CS), hyaluronic acid (HA) and poly(lactic-co-glycolide acid) (PLGA) (Liang et al., 2015).

CS is a cationic polysaccharide, derived from chitin, widely investigated as a carrier in the treatment of respiratory diseases such as TB and lung cancer (Rasul et al., 2020), owing to its outstanding biodegradability, biocompatibility, and mucoadhesive properties (Grenha et al., 2010). The molecular weight of CS is a critical issue to be considered since toxicity problems were observed in the range of 190–310 kDa (Bitencourt et al., 2015). For instance, a promising non-toxic carrier was developed from 50-190 kDa CS encapsulating the antitubercular drug isoniazid by spray drying (SD) and its drug retention was enhanced by crosslinking CS with tripolyphosphate (Oliveira et al., 2017). In addition, CS has low solubility in acidic and alkaline media. To face this issue, a soluble derivate of CS (N,N,N-trimethyl chitosan chloride) was proposed as an alternative CS-based carrier by Pardeshi et al., 2020. In this study, N,N,N-trimethyl chitosan chloride was bound to the bronchodilator etofylline, crosslinked with tripolyphosphate, and mannosylated to target mannose receptors in the alveolar region and improve the therapeutic efficiency of the drug (Pardeshi et al., 2020).

HA, a non-sulfated glycosaminoglycan naturally present in the lungs, has protective and reparative roles (Surendrakumar et al., 2003). HA is widely employed in pulmonary drug delivery for its excellent mucoadhesive properties and ability to delay phagocytosis (Liang et al., 2015). For example, the incorporation of insulin in HA-based dry powders could extend its retention in beagle dogs (Surendrakumar et al., 2003) and the encapsulation of SS in HA MPs increased its retention time in lungs thanks to the enhanced mucoadhesion (Li et al., 2017). The use of low molecular weight HA as a microcarrier has also been tested in gene

therapy to deliver inhalable naked plasmid DNA (pDNA) powders, obtained by spray freeze-drying (SFD). These powders allowed for high gene expression and had a MMAD of around 5 μm, pointing out low molecular weight HA as a suitable excipient for inhalation formulations (Ito et al., 2019).

Sustained release of inhaled drugs can be obtained by exploiting the properties of PLGA. This synthetic, biodegradable, biocompatible polymer can easily be engineered by tuning its composition, molecular weight, and chemical structure (Ungaro et al., 2012b). An *in vitro* investigation demonstrated that porous PLGA MPs allowed for sustained release of budesonide and showed higher lung uptake efficiency than non-porous ones (Oh et al., 2011). The tendency of PLGA MPs to undergo phagocytosis can be exploited to target alveolar macrophages in patients affected by TB, inducing the autophagy of infected macrophages without altering their cytokine profile and thus causing a significant reduction of TB bacteria replication in the lungs (Lawlor et al., 2016). Furthermore, PLGA microcarriers were combined with an active metabolite of vitamin A (all *trans*-Retinoic acid) which produced an antibacterial effect by stimulating the inflammatory response in TB-infected mice (O'Connor et al., 2019).

3.2.2. Large porous particles

Although common respirable MPs (1–5 μm) can reach deep airways of the lungs, these particles are rapidly engulfed by alveolar macrophages. To increase the residence time of inhaled drugs, Edwards et al. (1997) introduced LPPs, also referred to as AIR® or ARCUS™ technology (Healy et al., 2014), as a new attractive carrier-free delivery system. The combination of large geometric diameters (5–30 μm) and low mass densities (<0.1 g/cm^3) makes LPPs both not engulfable by macrophages and aerodynamically performant, i.e., with an optimal d_a (Dunbar et al., 2002; Duong et al., 2021).

Generally, LPPs are prepared by multiple emulsions (water-in-oil-in-water) and then freeze-dried (Giovagnoli et al., 2007; Kim et al., 2012; Patel et al., 2014; Ungaro et al., 2006). The desired porosity is obtained through porogens, such as ammonium bicarbonate, poly(vinyl pyrrolidone) (PVP), and cyclodextrins, which are added to the primary solution (Liang et al., 2015). For example, PLGA LPPs produced by double emulsion using hydroxypropyl beta-cyclodextrin (HPβCD) as porogen showed good therapeutic results even at low drug doses in *in vivo* tests on mice (Ungaro et al., 2009). Other techniques, including SD (Chvatal et al., 2019; Garcia-Contreras et al., 2007; N'Guessan et al., 2018), supercritical fluid technology (SCF) (Dhanda et al., 2013), and single emulsion (Ni et al., 2017a), have been employed to generate LPPs. A comparison between spray-dried non-porous particles and LPPs, both loaded with the anti-inflammatory drug meloxicam (for CF and COPD treatment), showed a remarkable superiority of LPPs in terms of aerodynamic performance (Chvatal et al., 2019). In another study, SFD was performed to obtain inhalable PLGA LPPs loaded with the vasodilator sildenafil citrate (ratio PLGA to drug 3:1) for the treatment of PAH. The formulation containing 1 % of polyethylenimine (PEI), and 1 % of polyvinyl alcohol (PVA) exhibited the best outcomes in terms of MMAD, FPF, and entrapment efficiency (Shahin et al., 2021). Furthermore, LPPs of mannitol and SS with MMAD around 4.4 μm were produced through ultrasonic SFD in a recent research, pointing out that good aerodynamic properties are obtained at low solid concentration and feed flow rate (Pasero et al., 2023).

One of the main issues of LPPs is the shortage of controlled drug release (Liang et al., 2015) due to the low encapsulation efficiency of several drugs and the problematic optimization of pore size (Rawat et al., 2008). In a study conducted by Rawat et al. (2008) an increasing burst release of heparin was observed for PLGA microspheres with progressively higher porosity. A possible solution was proposed by Zhang *et al.* through a novel formulation of PLGA LPPs containing the porogen PVP (Zhang et al., 2020). The new LPPs had a heterogeneous porous structure with unaltered internal porosity and reduced external porosity, limiting the *in vitro* burst release, and maintaining the LPPs' aerodynamic properties (Zhang et al., 2020).

3.2.3. Swellable microparticles

Swellable MPs have been proposed as an alternative carrier-free strategy for pulmonary drug delivery by El-Sherbiny et al., 2010, showing better control of drug release compared to LPPs (Liang et al., 2015). Swellable MPs are moisture-sensible powders, characterised by a geometric diameter of around 0.5–5 μm in the dry state, which is obtained mainly by spray-dried CS (El-Sherbiny et al., 2010; Wang et al., 2021b; Zhang et al., 2018a) or alginate (El-Sherbiny and Smyth, 2010). The uniqueness of these MPs is their increased size in the humid environment of the lungs, which renders them too large for phagocytosis (El-Sherbiny et al., 2015).

A novel technology is the incorporation of drug-loaded NPs into swellable MPs, merging the advantages of NPs and swellable MPs to obtain an increased sustained drug release. For example, El-Sherbiny *et al.* encapsulated PLGA NPs in polyethylene glycol (PEG)-CS microspheres (El-Sherbiny and Smyth, 2012). More recently, Ni *et al.* developed a nanocrystal-in-MP technology, based on chitosan swellable MPs loaded with cinaciguat nanocrystals for PAH treatment (Ni et al., 2017b).

3.3. Nanoparticles

Over the past decades researchers have emphasized the use of NPs to entrap, adsorb, encapsulate, or attach drugs, developing new drug delivery systems (De Jong and Borm, 2008; Sung et al., 2007). In the pulmonary delivery field, the most widely studied NPs are liposomes, solid lipid NPs, polymeric NPs, dendrimers, inorganic NPs, and nanocrystals (Fig. 3) (Pramanik et al., 2021). Extracellular vesicles have also emerged recently as innovative nanocarriers and biomarkers of lung diseases (Holtzman and Lee, 2020). The small particle size of NPs (<1 μm (Scherließ et al., 2022)) provides a higher surface area to volume ratio in comparison to MPs, guaranteeing increased drug concentration and bioavailability since alveolar clearance is reduced (Muralidharan et al., 2015; Praphawatvet et al., 2020). Moreover, NPs are characterised by high solubility, significant cellular uptake, mucus penetration, and longer lung half-time (Muralidharan et al., 2015). Although these advantages make NPs suitable for treating both pulmonary and systemic diseases, several issues should be considered (Sung et al., 2007). First, exhalation of NPs can occur owing to their small size thus limiting their therapeutic effectiveness (Sung et al., 2007). Another concern related to using NPs in inhalation therapy is their potential toxicity since NPs can lead to lung inflammation and fibrosis (Pramanik et al., 2021). Furthermore, the high surface energy is responsible for the significant cohesive forces among NPs and their reduced flowability, which makes the use of larger carriers widespread for the pulmonary delivery of nano-sized drugs (Praphawatvet et al., 2020). Although drug-loaded NPs are usually incorporated in a microparticulate sugar matrix (Zillen et al., 2021), alternative delivery systems, including nano-in-MPs and effervescent NPs, have been investigated.

3.3.1. Polymeric nanoparticles

As already discussed for MPs, polymers such as CS, HA, and PLGA are widely employed also in the formulation of NPs for pulmonary delivery for their biodegradability profile, cell targeting ability, and escaping of the immunity system (Liang et al., 2015). For example, PLGA NPs have been investigated for the pulmonary delivery of glucocorticoids (Rigo et al., 2017), antibiotics (Ungaro et al., 2012a), and vaccines (Scherließ and Janke, 2021). Polymeric NPs can vehiculate drugs by adsorbing, chemically conjugating or encapsulating them, and, according to their structure, they are classified into nanocapsules and nanospheres (Pramanik et al., 2021; Vauthier and Bouchemal, 2009). Nanocapsules consist of a liquid or semisolid core surrounded by a solid shell, while nanospheres are full solid matrices, generally spherical (Vauthier and Bouchemal, 2009). The main issues related to the therapeutic inhalation

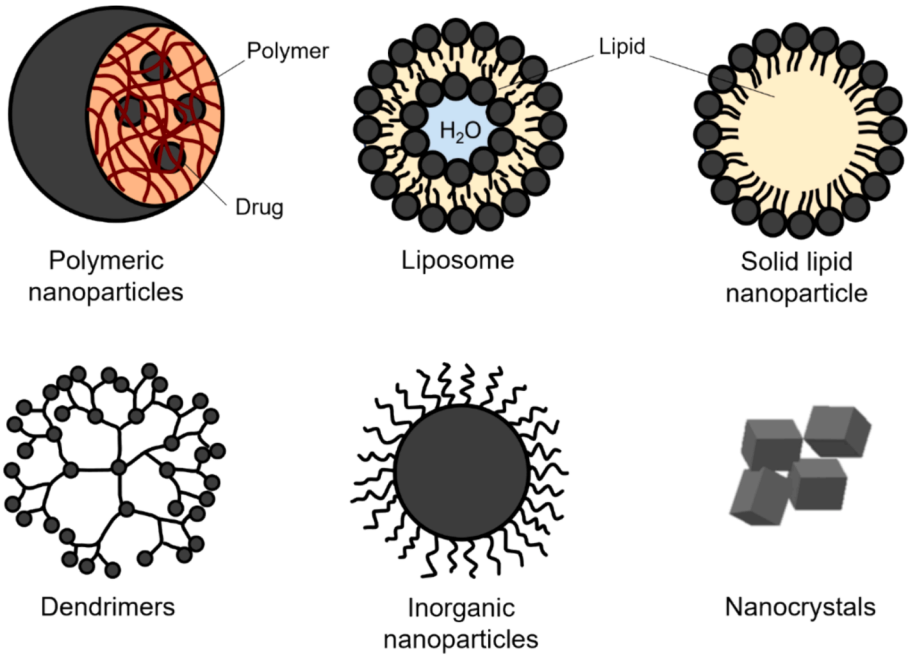

Fig. 3. Representation of the structure of the most employed NPs.

of polymeric NPs alone are their low flowability and relevant exhalation (Liang et al., 2015). Therefore, in the pulmonary delivery field polymers are mostly used to encapsulate NPs or to form a matrix where NPs are dispersed and that is rapidly dissolved after inhalation (Muralidharan et al., 2015). Further details about this technology, namely nano-in-microparticles, are given in section 3.3.8.

3.3.2. Liposomes

Liposomes are submicron synthetic vesicles, discovered by Bangham *et al.* in 1965 (Mehta et al., 2020) as excellent drug carriers, capable of sustained or prolonged release of active lung agents (Nirale et al., 2009). Liposomes consist of a liquid core surrounded by a single (or double) lipid bilayer, whose composition can be varied to modulate liposome surface and physical properties (Ibaraki et al., 2020). According to the number of bilayers and size, liposomes are divided into small unilamellar vesicles (SUVs; 20–100 nm), large unilamellar vesicles (LUVs; 100 nm), giant unilamellar vesicles (GUVs; > 1000 nm), and multilamellar vesicles (MLV; > 500 nm) (Mehta et al., 2020). Among them, the optimal size range of liposomes for drug delivery is 50–500 nm (Kuzmov and Minko, 2015). The peculiar structure of liposomes is suitable for encapsulating hydrophobic, hydrophilic, or amphipathic drugs, including cytotoxic agents, anti-asthma drugs, antioxidant agents, and systemic drugs (Zeng et al., 1995). The first approved pharmaceutical liposomal product for pulmonary applications is Alveofact® (Lyomark Pharma, Oberhaching, Germany), a synthetic surfactant suspension for treating respiratory distress syndrome (Mansour et al., 2009). A successful application of this product can be found in a recent study investigating coating of PEI polyplexes with Alveofact® for small-interfering RNA (siRNA) pulmonary delivery, where enhanced mucus penetration and gene silencing were observed for coated PEI polyplexes with respect to the uncoated ones (Baldassi et al., 2022). Another example of a marketed surfactant is Curosurf® (Chiesi, Parma, Italy), which is intratracheally administered to treat respiratory distress syndrome (Schulz et al., 2021). Furthermore, in 2018 FDA promoted the approval of Arikayce® (amikacin liposome inhalation suspension), a liposomal formulation developed by Insmed Pharmaceuticals (New Jersey, USA) (He et al., 2022). Arikayce® formulation is based on the phospholipid dipalmitoylphosphatidylcholine (DPCC) and cholesterol and has been studied in the treatment of CF and various lung infections, showing prolonged drug release, and reduced systemic exposure (He et al., 2022; Mehta et al., 2020).

Liposomal nanocarriers are extremely biocompatible since they are prepared from substances endogenous to the lungs such as phospholipids, naturally present in lung surfactants (Mehta et al., 2020). Among the different phospholipids, liposomes mainly comprise lecithins, phosphatidylethanolamines, sphingomyelins, phosphatidyl glycerol and phosphatidylserines (El-Sherbiny et al., 2015). Both aqueous (Valle et al., 2018) and dry powder formulations (Desai et al., 2003) of liposomes are available; however, the latter approach usually results in higher stability than the former one. Dry powders of liposomes are generally obtained by freeze-drying followed by jet-milling (Desai et al., 2003) or SD (Chougule et al., 2008; Zhu et al., 2019). In a study conducted by Chougule et al. (2008), spray-dried respirable particles of dapsone-loaded liposomes demonstrated higher MMAD and FPF and prolonged *in vitro* drug release compared to dapsone-loaded lactose particles. In another study, folic acid-conjugated doxetacel liposomes were spray-dried with mannitol and L-leu to produce dry powders for lung cancer treatment, resulting in higher cellular uptake, toxicity, and therapeutic effect in comparison to nebulized liposomes (Zhu et al., 2019). To produce liposomal powders with high porosity and aerodynamics, also SFD can be used. Ye et al. (2017) formulated inhalable clarithromycin liposomal powder with aerosolization efficiency of over 85 % and FPF up to 50 %. In this study, the action of sucrose and mannitol as excipients was investigated, highlighting sucrose as an effective lyoprotectant and crystalline mannitol as a good moisture-protectant.

3.3.3. Solid lipid nanoparticles

Since the early 1990s solid lipid NPs (SLNs) have been investigated as carriers for lipophilic drugs (Pramanik et al., 2021). SLNs have a size ranging between 40 nm and 1000 nm (Weber et al., 2014). They are characterized by a phospholipidic monolayer encapsulating a solid hydrophobic core (El-Sherbiny et al., 2015), that can be prepared from endogenous substances guaranteeing high tolerability in lung airways (Weber et al., 2014). Due to the high physical stability and deep-lung deposition SLNs can be used for pulmonary drug delivery, allowing for the prolonged release of drugs and ensuring high therapeutic efficacy and better patient compliance (Weber et al., 2014). The preparation of

these particles is often solvent-free and easy to scale up (Weber et al., 2014) since high-pressure homogenization and microemulsion are the most used techniques (Müller et al., 2000).

SLNs are commonly delivered by nebulizers (Liu et al., 2008; Pandey and Khuller, 2005) and DPIs (Li et al., 2021; Wang et al., 2021a; Yeganeh et al., 2020), and even if the former method is the most widespread owing to the good stability of SLNs under nebulization (Healy et al., 2014), several studies also investigated dry formulations of lyophilized SLNs. For example, Yeganeh et al. (2020) spray-dried lactose with SLNs carrying a mixture of the antifungal agent Amphotericin B and the solubility enhancer phospatidylglycerol (Yeganeh et al., 2020). In another study, freeze-drying technology was applied to obtain powders of curcumin-loaded SLNs to treat COPD (Li et al., 2021). Furthermore, dry powders of SLNs enclosing siRNA were successfully obtained through the thin film freezing (TFF) technology (Wang et al., 2021a) and SD (Zimmermann et al., 2022).

3.3.4. Dendrimers

Dendrimers are macromolecules, such as sugars, nucleotides, or amino acids, constituted by a central core with branches of different terminal active groups (Pramanik et al., 2021). These NPs can load drugs inside their cavity or linked to their surface functional groups, which can easily be modified to increase the number of drugs potentially carriable (Luo et al., 2021). This versatility, the high biocompatibility, and the small size (4–20 nm) of dendrimers make them suitable carriers for pulmonary delivery, especially when the therapeutic agents are targeted to the alveoli (Luo et al., 2021; Yhee et al., 2016). However, due to the extremely short retention of these nanocarriers in lungs (Yhee et al., 2016) dendrimers are usually coupled with other molecules to be employed as carriers (Kuzmov and Minko, 2015).

Polyamidoamine (PAMAM) dendrimers have been investigated in the treatment of TB (Rajabnezhad et al., 2016) and to deliver siRNA to the lungs (Bielski et al., 2017; Bohr et al., 2020). SD PAMAM dendrimers and mannitol resulted in microparticles with high entrapment efficiency and favourable aerodynamic performance. Moreover, the complexation of siRNA-PAMAM dendrimers with triphenylphoshonim ion has been suggested to enhance mitochondrial targeting and cellular internalization (Bielski et al., 2017). Promising outcomes were also observed with POxylated Polyurea dendrimers, combined with the nano-in-MP technology, which is detailed below (Restani et al., 2018, 2016). For example, dendrimers loaded with ibuprofen were encapsulated into inhalable CS MPs, showing sustained release and high cellular uptake (Restani et al., 2016).

3.3.5. Inorganic nanoparticles

NPs can be obtained from inorganic materials, such as gold, silver, iron, and silica (Yhee et al., 2016), ensuring a high surface/volume ratio, stability, good optical and magnetic properties, biocompatibility, and hydrophilicity (Pramanik et al., 2021). Besides these advantages, the use of inorganic NPs is still restricted owing to safety concerns (García-Fernández et al., 2021) and their dose and exposition should be strictly regulated to overcome toxicity issues (Abdelaziz et al., 2018). In the pulmonary delivery field, the most investigated inorganic NPs are metal, magnetic, and mesoporous silica NPs, although metal ones are less used due to toxicity problems (Abdelaziz et al., 2018). Mesoporous silica NPs (MSNs) have been suggested as alternative carriers in inhalation therapy owing to their ability to carry the active agent either on their surface or inside pores (Abdelaziz et al., 2018). Liquid and spray-dried formulations of MSNs are commonly used for nebulisers and DPIs, respectively (García-Fernández et al., 2021). Magnetic NPs (MNPs) have been gaining increasing interest (Abdelaziz et al., 2018) thanks to their capacity to react to the presence of an external magnetic field. This property could potentially be exploited to drive NPs directly to the diseased site of the lung, thus preventing undesired off-target effects (Ebrahimi et al., 2021). In addition to standard iron oxide Fe_3O_4 or gamma-Fe_2O_3 MNPs, MNPs can be surface engineered, i.e., by coating with PLGA to improve their aerosolization performance (Verma et al., 2013). Alternatively, superparamagnetic iron oxide NPs can produce magnetic aerosols (nanomagnetosols), allowing controlled drug targeting. The delivery of nanomagnetosols was investigated in mice, observing that particle deposition in the lungs was higher when a target-directed magnetic gradient field was applied (Dames et al., 2007).

3.3.6. Nanocrystals

Nanocrystals represent a suitable system for the pulmonary delivery of high-dose drugs, which are usually not administered by inhalation. This technology offers a large surface area, a high dissolution rate, and good stability (Pramanik et al., 2021; Scherließ et al., 2022). Nanocrystals are obtained either by bottom-up (precipitation, SD) or top-down (high-pressure homogenization, media milling) techniques (Scherließ et al., 2022). Wet milling and SD have also been combined to generate dry powders of curcumin nanocrystals with appropriate physical and chemical stability (Hu et al., 2015). In a study conducted by Khatib et al. (2020) nanocrystals of the antibiotic ciprofloxacin were encapsulated in liposomes by SD, using sucrose as a lyoprotectant. The use of a crystalline form of the antibiotic allowed for the slow release of the drug, hence reducing the number of inhalations required per day (Khatib et al., 2020). In another study, the advantages of nanocrystals were combined with the favourable aerodynamic properties of MPs. In fact, HA MPs were loaded with budesonide nanocrystals through an SD process. It was observed that such particles exhibited an extended pharmacological effect than the analogous nanocrystalline drug suspension (Liu et al., 2018). This novel formulation was based on producing inhalable nanocrystals-embedded MPs (NEP), constituted by nanocrystals embedded in a matrix of excipients. The use of NEP involved optimal d_a, thus allowing deep-lung deposition. Moreover, the contact with lung walls enhanced the redispersion of NEP and the release of individual nanocrystals, pointing out this strategy as a potential approach to deliver poorly soluble drugs via inhalation (Chen et al., 2021).

3.3.7. Extracellular vesicles

Among the various drug carriers, extracellular vesicles (EVs) have recently attracted much attention due to their outstanding ability to be absorbed into host tissues and transport cargo such as nucleic acids, lipids, and proteins (Holtzman and Lee, 2020). EVs are exocytotic NPs, consisting of phospholipid bilayers, which play a leading role in intercellular communication, inflammatory responses, cancer initiation and progression (Holtzman and Lee, 2020; Limongi et al., 2021; Wu et al., 2017). The last Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines were published in 2018 by the International Society for Extracellular Vesicles to assist researchers and clinicians in standardising EVs' isolation and characterization methods (Théry et al., 2018). MISEV classifies EVs in apoptotic bodies (ABs), microvesicles (MVs), and exosomes, according to the vesicle's size, biochemical composition, and EVs' cellular origin (Carnino et al., 2021). While ABs are the largest EVs (1000 nm–5000 nm) produced during cells apoptosis, microvesicles (100 nm-1000 nm) are formed by subsequent budding and pinching of the cell membrane. Exosomes are multivesicular bodies of size about 30 nm-100 nm generated inside the cell and secreted following their fusion with the membrane (Carnino et al., 2021). EVs are important in pulmonary physiopathology tuning cellular homeostasis, proliferation, and migration (Zhou et al., 2022). Airways cells release EVs with proinflammatory effects in the lung and circulation to reach target cells in distant organs and tissues (Margaroli et al., 2022; Wahlund et al., 2017). Since many authors documented the role of EVs in respiratory diseases such as COPD (O'Farrell and Yang, 2019), idiopathic pulmonary fibrosis (Fujita, 2022), PAH (M. Zhang et al., 2018b), and asthma (Nagano et al., 2019) these cellular-derived lipid NPs are excellent candidates for pulmonary delivery of therapeutics (Carnino et al., 2021; Holtzman and Lee, 2020). A study conducted by Popowski et al. (2022) compared the aerosolization performances of exosomes and

liposomes in terms of distribution, cellular uptake, and mucus penetration. Higher lung retention and lower mucoadhesion were exhibited by exosomes over liposomes and dry exosomes were more efficiently distributed than nebulised ones (Popowski et al., 2022). Moreover, pulmonary exosomes represent the most suitable carrier for microRNA (miRNA) administration (Holtzman and Lee, 2020). miRNA is a non-coding RNA whose therapeutic potentiality is notoriously undermined by stability issues (Li et al., 2022). Being miRNA a natural component of EVs, EV-associated miRNAs have been introduced as an innovative route for miRNA delivery (Carnino et al., 2021; Holtzman and Lee, 2020). A significant contribution to the development of EV-miRNA was given by Zhang *et al.*, who investigated a novel way of loading miRNA into exosomes by means of a modified calcium chloride transfection (Zhang et al., 2017).

Lung exosomes DPIs are currently used to develop inhaled vaccines to treat several infectious respiratory diseases, including COVID-19 (Popowski et al., 2022). The application of EVs to pulmonary immunisation has also been tested by Mehanny et al. (2022), who produced spray-dried pneumococcal MVs, loaded into lactose MPs. Such particles successfully induced the release of proinflammatory cytokines from human primary immune cells, thus demonstrating the enormous potentiality of EVs in delivering inhaled vaccines (Mehanny et al., 2022).

3.3.8. *Nano-in-microparticles*

Despite their significant advantages, individual NPs are not suitable for the pulmonary administration of drugs and require microcarriers to be delivered. Alternatively, NP can be spray-dried with other substances to form micron-scale structures, the so-called nano-in-microparticles (NiMs). This strategy has been applied to various NPs, including polymeric NPs (Party et al., 2021; Porsio et al., 2018), nanocrystals (Chen et al., 2021; Liu et al., 2018), and POxylated Polyurea dendrimers (Restani et al., 2018, 2016).

NiMs technology includes nanocomposites and porous NP-aggregate particles (PNAPs), also known as Trojan particles (Fig. 4). Nanocomposites are MPs formed by drug-loaded NPs embedded in a micronized sugar matrix which, after inhalation, dissolves and releases individual NPs in the lumen of the airways (Fig. 4a) (Miranda et al., 2018). SFD is considered the best technique to produce nanocomposites with optimal aerodynamic performances and good reconstitution of the single NPs (Ali and Lamprecht, 2014), but also SD, micronization, and SCF are employed (Teymouri Rad et al., 2019). By contrast, PNAPs are constituted by NPs organised in large porous or hollow structures (Sung et al., 2007), where they are kept together by physical forces (i.e., capillary and van der Waals forces) or incorporated in a polymeric matrix. PNAPs are primarily prepared from spray-dried solutions of NPs and excipients, including lactose, albumin, hydroxypropyl cellulose, and phospholipids (Hadinoto et al., 2006). Once inhaled, the matrix of such nano aggregates breaks up in single NPs, thus allowing for sustained release (Fig. 4b) (Liang et al., 2015). PNAPs combine the advantages of NPs and LPPs, guaranteeing good flowability, deep-lung deposition, phagocytosis avoidance (Yang et al., 2013) and reduced burst release, leading to controlled drug delivery (Anton et al., 2012).

PNAPs have been proposed by Tsapis et al. (2002), who successfully developed nano aggregates of silica and polystyrene (PS) NPs. They observed that the final structure of PNAPs was mainly dependent on the presence of excipients and on the concentration of NPs, while the ability to form PNAPs was independent of the size and nature of NPs. To overcome the non-biodegradability limitations affecting silica and PS nano aggregates, Hadinoto et al. (2006) synthesised polyacrylate Trojan particles by SD. In this study, they compared silica, PS, and polyacrylate PNAPs, observing that the dependence of the degree of hollowness on the concentration of NP was related to the nature of NPs themselves. Moreover, it was highlighted that a threshold concentration of NPs should be reached to generate porous structures. The possibility of achieving sustained release of therapeutic agents was confirmed by a study conducted by Yang et al. (2013) on pulmonary peptide delivery, where SFD was investigated as an alternative method to preserve the stability of peptides. The same technique was performed by Cheow et al. (2011) to prepare poly(caprolactone) NPs loaded with the antibiotic levofloxacin. According to this study, the structure of nano aggregates strongly depended on the type of SFD adjuvant; in fact, the use of mannitol led to a final porous matrix, while PVA acted as a coating agent (Cheow et al., 2011). In a recent study, carbohydrate-based lipid Trojan particles were further developed to analyse the influence of the excipient in PNAPs formulations. Among the examined carbohydrates (i.e., raffinose, trehalose, sucrose, lactose, and mannitol), raffinose allowed for better aerodynamic properties and satisfactory NP dispersibility (Umerska et al., 2020).

3.3.9. *Effervescent nanoparticles*

Although the effervescent technology has been extensively used in the pharmaceutical industry for more than 200 years, the first attempt to produce effervescent dry powders for pulmonary delivery was only made in 2007 (Ely et al., 2007). This technology is based on effervescent particles loaded with drug-based NPs commonly obtained from a formulation including sodium carbonate, citric acid, ammonium hydroxide, and ammonia as a buffer (Muralidharan et al., 2015). Ely et al. (2007) compared the effect of the effervescent carrier on common lactose particles, observing a faster drug release in the first case. This study also demonstrated that the effervescent technology could ensure the active release of therapeutic agents, thus enhancing drug dissolution and the dispersion of NPs (Ely et al., 2007). In another study, effervescent carriers containing doxorubicin NPs were tested in lung cancer bearing mice (Roa et al., 2011), resulting in a longer survival time than

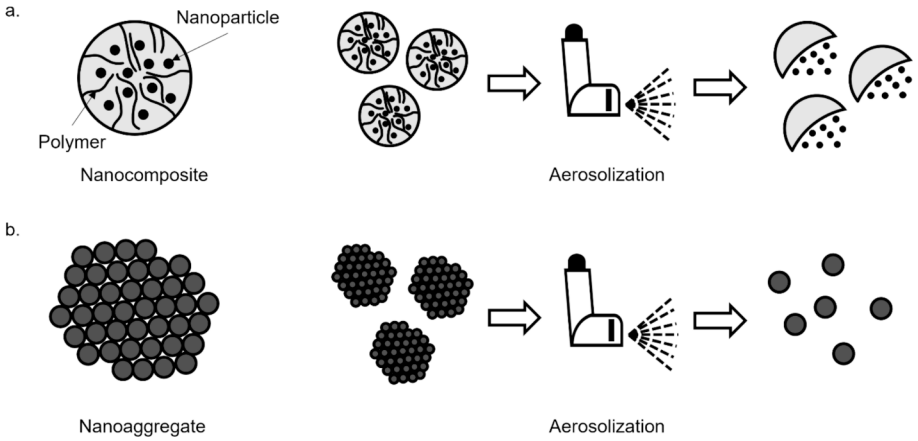

Fig. 4. Aerosolization process of (a) nanocomposites and (b) nanoaggregates (PNAPs).

mice treated with the non-effervescent therapy. A recent investigation explored the use of FDKP as an effervescent carrier, highlighting the possibility of enhancing deep-lung deposition and limiting the action of macrophages by means of drug-loaded FDKP effervescent particles (Wang et al., 2020).

4. Engineering of inhalable particles

Particle engineering is a well-established technique to develop respirable particles with enhanced targeting ability, improved particle dispersion, longer residence time, and capable of bypassing the lung defence mechanisms (Scherließ et al., 2022). Generally, particle size, surface charge, and shape can be varied to achieve inhaled particles' desired aerosolization properties and deposition patterns. In adhesive drug-carrier blends, particle engineering is mainly exerted on the carrier, by surface roughness adjustment and addition of fine carriers. Another strategy is surface coating, which is widespread for adhesive blends and carrier-free formulations (Muralidharan et al., 2015). For example, coating PLGA NPs with either CS or PVA is an excellent way to enhance their transport in mucus (He et al., 2022). The above-mentioned LPPs, hollow particles, NiM particles, and effervescent NPs are examples of engineered particles and some approved pulmonary products based on engineered particles are TOBI Podhaler (Novartis, Basel, Switzerland), Bevespi Aerosphere (AstraZeneca, Cambridge, UK), Afrezza (Mannkind, CA, USA), and Inbrija (Acorda Therapeutics, New York, USA) (Clark, 2022). The main techniques used to engineer carrier-free particles are reported in section 4.2.

4.1. Engineering of adhesive mixtures

4.1.1. Modification of carrier roughness

In adhesive blends, the surface roughness of carriers plays an important role in drug detachment since it influences the strength of binding between carrier and drug. Several studies examined the influence of carrier roughness on aerosolization properties, leading to contradictory results. Zeng et al. (2000) demonstrated that elongated and smoothed lactose particles had superior aerosolization performances than spherical ones. Similarly, Iida et al. (2003) observed that smoothing lactose carriers with an aqueous ethanol solution reduced the adhesion forces between drug and carrier, increasing the FPF and enhancing the detachment of drugs. In another study, Ferrari et al. (2004) applied the same wet-smoothing process to lactose obtaining improved packing of powders in DPI, other than higher FPF compared to non-treated lactose. By contrast, an opposite effect was shown by Dickoff *et al.*, who observed weak drug detachment from lactose carriers treated with ethanol solutions, pointing out that the effect of the surface treatment strongly depended on the conditions used (Dickhoff et al., 2006).

Moreover, Kaialy et al. (2012) obtained enhanced DPI performances using milled lactose with an elongated shape and rough surface. These contrasting results could be explained by the uncertainty that affects the definition of "smooth" and "rough" (Renner et al., 2017) since smooth, micro-metered topography and nano-metered topography carriers must be distinguished, according to the size of their roughness (Fig. 5) (Peng et al., 2016). The first class defines surfaces with few irregularities, the micro-metered topography refers to pores' size similar to the micronized drug's size, while the nano-metered topography includes pores smaller in size than the drug (Shalash et al., 2015). A study conducted on mannitol by Littringer et al. (2012) indicated the last category as the most appropriate one for drug delivery since the balance between drug adhesion and detachment was achieved, while for smooth surfaces or surface cavities adhesion forces were predominant. Renner et al. (2017) also confirmed the superiority of nano-scaled roughness, showing the effect of carrier roughness on the aerosolization performance of two blends containing formoterol fumarate dihydrate and budesonide, respectively.

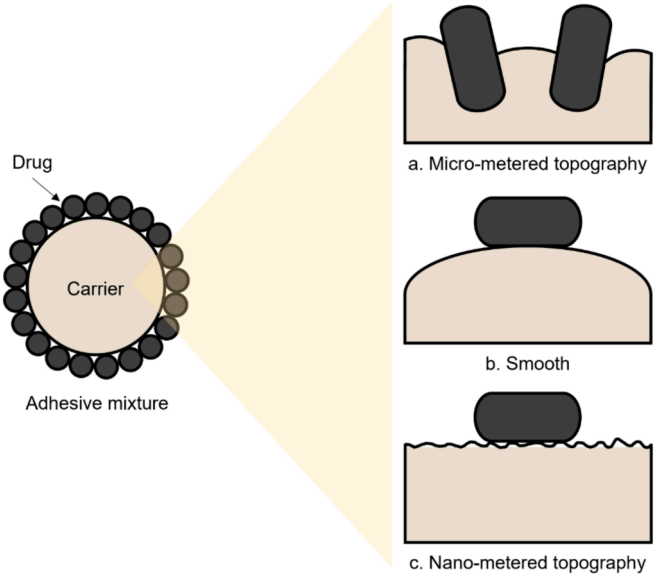

Fig. 5. Graphic representation of the types of carrier surface roughness. (a) Micro-metered topography. (b) Smooth. (c) Nano-metered topography. Modified from (Peng et al., 2016).

4.1.2. Coating of the carrier surface

The adhesion between carrier and drug can also be reduced by coating the carrier surface with force control agents, including polymers, MgSt, and L-leu. This process can be either wet or dry (Zhou and Morton, 2012). Iida et al. (2005) applied the wet fluid-bed coating to coat lactose carriers with a solution of lactose and hydroxypropyl methylcellulose, which gave better aerosolization properties than non-treated lactose (Iida et al., 2005). Another approach, known as "wet smoothing", consists of coupling particle smoothing with coating and is commonly performed in the presence of low surface free energy substances, such as MgSt (Ferrari et al., 2004; Young et al., 2010). Comparing the aerosolization properties of three blends prepared from beclomethasone dipropionate (BDP) and (i) untreated lactose, (ii) wet-smoothed lactose, (iii) wet-smoothed lactose with the addition of MgSt, a higher FPF with modified lactose carrier (ii, iii) stood out and this effect was remarkably higher in (iii) than in (ii) (Young et al., 2010). SD was also employed to wet-coat lactose particles with ethyl cellulose (EC) and polyvinylpyrrolidone (PVP), observing superior aerosolization performances in the presence of EC-coated lactose, which has lower adhesion strength and surface free energy than PVP-coated lactose (Traini et al., 2012).

Since wet coating is generally tricky, energy, time-consuming, and potentially dangerous, the favourite option is a mechanical dry coating, where coating agents are bound to the large carrier surface through physical and chemical interactions (Zhou and Morton, 2012). Dry coating is commonly performed by mechanofusion, high shear mixing, fluid energy mill, magnetically assisted impact coating and Theta-composer technique (Sharma and Setia, 2019; Zhou and Morton, 2012). A wide range of applications of these techniques has been extensively reviewed by Sharma and Setia, 2019. In a recent study, Hertel *et al.* produced MgSt-coated mannitol carrier particles with a high shear mixer demonstrating that MgSt could act as both smoothing and force control agent even at low concentrations, resulting in reduced particle interactions and increased FPF (Hertel et al., 2020b). Moreover, a comparison between high shear mixing and mechanofusion highlighted the first approach as the preferred coating method for DPI carriers since more complete coating and a more suitable speed of drug detachment could be achieved (Bungert et al., 2021).

4.1.3. Addition of fine carrier particles

Another strategy to improve the aerosolization properties of a blend is the addition of fine carrier particles (fines) to coarse ones to increase FPF while maintaining good flowability (Malcolmson and Embleton, 1998). These ternary agents should be comparable in size to the active compound or, at least, smaller than the coarse carrier. Although the mechanism behind fine carrier particle action has not yet been established, various hypotheses have been developed (Peng et al., 2016). Hersey (1975) introduced the active-sites theory, which states that fine carrier particles preferentially bind highly adhesive regions of carriers, called active sites, leaving weaker sites for the drug, and promoting drug delivery. The active site theory was recognised as the prevalent mechanism of action of fines in a study on SS and lactose adhesive blends (Zeng et al., 1998).

Similarly, Guchardi et al. (2008) tested the effect of fine lactose particles (smaller than 10 μm) in a formulation of coarse lactose and formoterol fumarate. An increase in FPF was observed by adding fine lactose up to 5 % and this correlation appeared linear in the presence of MgSt. On the other hand, the absence of favourable effects for lactose concentrations over 5 % was attributed to the saturation of the active sites, endorsing Hersey's theory (Guchardi et al., 2008). Another hypothesis is the agglomeration theory, which attributes the higher FPF to the formation of drug-fines agglomerates, which are easily detached from the carrier after inhalation. Kinnunen et al. (2015) showed the formation of agglomerates of fine lactose and budesonide, pointing out this theory as the dominant mechanism. Conversely, according to Shur et al. (2008), the presence of fines affects the fluidisation properties of dry powders. The consequent increase of the aerodynamic drag force exerted to fluidise the particle bed may be responsible for enhancing drug detachment (Shur et al., 2008). A fourth theory, known as the buffering hypothesis, considers fines as a buffer, which protects coarse carriers from collision and limits drug losses during inhalation (Dickhoff et al., 2006).

Lastly, Grasmeijer et al. (2014) suggested that the synergic action of all the mechanisms mentioned above was responsible for the effect of fines, which also depended on the formulation, the size of the fine, the drug content, the mixing order, and the inhalation flow rate. They highlighted the importance of fines size, comparing "fine lactose fines" (FLF) of drug-like size with larger "coarse lactose fines" (CLF). In fact, the highest drug detachment was promoted by CLF. At the same time, the presence of FLF resulted in high press-on forces, which led to the formation of coherent fine particle networks on the carrier surface and strong drug-carrier adhesion (Grasmeijer et al., 2014).

A study conducted by Hertel et al. (2018) investigated the influence of the amount of fines on FPF in a lactose-based blend. They found out that the FPF increased with the percentage of fines in the formulation until a maximum value, and then decreased again or reached a plateau. Moreover, the study revealed that this trend depended on carrier size and type of inhaler (Hertel et al., 2018). A similar behaviour was observed for a mannitol-based blend, where adding mannitol fines increased the FPF until a plateau was reached. However, the maximum FPF was obtained only in the presence of a large amount of mannitol fines. In this study Hertel *et al.* (2020) also analysed the combined action of mannitol fines and MgSt, concluding that the increase in FPF was mainly ascribable to the presence of MgSt (Hertel et al., 2020b).

4.2. Engineering of carrier-free particles

Carrier-free particles with a size appropriate for pulmonary delivery can be produced through top-down or bottom-up techniques. The former refers to forming micronized particles from larger ones, while the latter refers to generating particles from solutions (Muralidharan et al., 2015). Milling is a traditional top-down technique to obtain MPs and NPs. The most employed variant is air jet milling, also called fluid energy milling, which is either a dry or wet process that impacts large particles with compressed air to obtain smaller particles (Chaurasiya and Zhao, 2021; Muralidharan et al., 2015). Milled particles are usually cohesive, highly charged, sensitive to moisture, and challenging to process downstream (Rehman et al., 2004). Therefore, this method is not optimal for obtaining dry powders for inhalation despite being low-cost, reproducible, and straightforward. Alternative techniques have been introduced to produce dry particles, such as SD, SCF, SFD, and TFF. Specifically, freezing-based technologies have been gaining growing attention due to the low temperatures involved, which are potentially suitable for thermosensitive substances (Overhoff et al., 2009). Some examples of DPI formulations recently produced by SD, SCF, SFD, and TFF are listed in Table 1.

4.2.1. Spray drying

SD is widely used to produce and dry particles in a single step (Jain et al., 2020). During this process, a solution is divided into fine droplets by atomisation under high pressure; droplets are then instantaneously dried by a hot stream of air or nitrogen (Pramanik et al., 2021) and collected from a cyclone (Duong et al., 2021). The main parameters affecting the properties of spray-dried particles are the solute concentration, the flow rate of drug solution and hot medium, the atomising pressure, and the inlet temperature (Muralidharan et al., 2015). Being a one-stage method, SD is more convenient and cost-effective than traditional milling. Owing to these advantageous features, SD has been widely used to produce engineered MPs with size, shape, and morphology suitable for pulmonary delivery as excellently provided by Vehring (2008). An application of SD in the pulmonary delivery field is represented by the PulmoSol™ technology (Nektar Therapeutics, San Francisco, USA), used to stabilise spray-dried insulin in an amorphous glass matrix (Healy et al., 2014). The use of SD was also reported by Park et al. (2013), who designed a dry formulation of the antibiotics vancomycin and clarithromycin by SD an alcoholic solution. Vancomycin aerosols showed good aerosolization performances, while co-SD enhanced the aerodynamic properties of clarithromycin aerosols with DPCC. A novel formulation devoid of organic solvent was then developed by Peng et al. (2017) to prevent safety issues concerning the employment of organic solvents in SD. In this study, inhalable mannitol-based carriers were prepared by SD from a solution of ammonium carbonate, a porogen agent, generating low-density nanoporous particles. Another recent study investigated the formulation of co-spray dried poly-L-lysine with L-leu to improve the aerosolization properties of DPIs for treating respiratory infections (Zhang et al., 2022). SD has also produced low-density porous particles, namely PulmoSpheres® (Novartis, Basel, Switzerland) and large porous particles (LPPs). In PulmoSpheres® the micronized drug can be dissolved, suspended, or dispersed in the excipient-based liquid before SD. The SD is performed

Table 1
Some examples of dry drug formulations engineered by SD, SCF, SFD, and TFF.

Drug	Additives	Technique	References
Adalimumab	L-leu, Phenylalanine, Glycine, Arginine	SFD	(Emami et al., 2019)
Gefitinib	Poly-L-Lactic Acid	SCF	(Lin et al., 2017)
Isoniazid	Chitosan, Tripolyphosphate	SD	(Oliveira et al., 2017)
Meloxicam	L-leu, Hyaluronic acid	SD	(Chvatal et al., 2019)
miRNA	Sucrose, trehalose, mannitol, L-leu	TFF	(AboulFotouh et al., 2024)
Niclosamide	L-leu, 1,2-Dioctadecanoyl-*sn*-glycero-3-phophocholine, Tween-80	SFD	(Zhang et al., 2023)
pDNA	Mannitol, L-leu	TFF	(Xu et al., 2023)
Remdesivir	Captisol®, mannitol, lactose, L-leu	TFF	(Sahakijpijarn et al., 2020a)
siRNA	Mannitol	SFD	(Liang et al., 2018)
Voriconazole	Mannitol, Tertiary butyl alcohol	SFD	(Liao et al., 2019)

from a perfluorooctyl bromide-in-water emulsion, and the result is a highly porous particles with size lower than 5 μm (Healy et al., 2014; Weers et al., 2019). As discussed by Weers et al. (2019), this technology can stabilise drugs by preserving their crystalline structure without affecting their aerosolization performance. Instead, LPPs are spray-dried from a solution and are bigger than 5 μm (Healy et al., 2014). Nano SD has also emerged as a promising approach to produce MPs or NPs with controlled size and high yields. Contrary to SD, nano SD employs vibrating-mesh atomizers for the spraying, laminar drying gas flow, and electrostatic precipitation to collect powders. The mesh pore size and vibration frequency, the viscosity of the sprayed solution, and the spray solution surface tension are responsible for the particle size distribution and the spray yields. A recent study on nano SD highlighted the potentiality of hydrochloric acid and hydroethanolic solvents in enhancing the spraying performance of mannitol and lactose, respectively (Almansour et al., 2022).

4.2.2. Supercritical fluid technology

SCF is a single-step method that produces spherical particles with diameters up to 3 μm and smooth surfaces (Jain et al., 2020; Pramanik et al., 2021; Rehman et al., 2004). It has been introduced as a competitive process owing to the low consumption of organic solvents and the possibility of obtaining optimal particle sizes for DPIs (Duong et al., 2021). Moreover, SCF guarantees high versatility and flexibility, allowing an enhanced control of process parameters such as pressure, solvent evaporation rate, and diffusivity of the supercritical fluid (Pasquali et al., 2008). This technique is particularly advantageous for forming crystalline powders with a minimum residue of solvents (Pramanik et al., 2021). During this process, the supercritical fluid (e.g., supercritical carbon dioxide, $scCO_2$) can act as a solvent or antisolvent (Jain et al., 2020). In the first case, the solute is dispersed into $scCO_2$, while in the second approach, the solute is dissolved in another solvent and $scCO_2$ behaves as an antisolvent. The latter alternative is the supercritical anti-solvent (SAS) process and is based on solute precipitation (Kim and Shing, 2008). Rehman et al. (2004) used SCF technology to manufacture inhalable dry powders of terbutaline sulphate, demonstrating that this technique was useful in modulating the polymorphism of the drug. The aerosolization properties of the corticosteroid budesonide were improved by combining $scCO_2$ processing and freeze-drying, in the presence of different additives. Particularly, the incorporation of the monoglyceride of hydrogenated palm oil was proposed as a cheaper alternative to DPCC (Miyazaki et al., 2017). Another study used the SAS method to formulate inhalable ipratropium bromide for treating asthma and COPD. The particle size and morphology could be successfully controlled by manipulating pressure, temperature, and type of solvent (Kim and Shing, 2008). Poly ($_L$-lactic acid) microspheres of gefitinib were also produced by SAS, highlighting the superior anti-cancer activity of such engineered particles compared to the raw drug (Lin et al., 2017). Similarly, SAS was employed to prepare respirable nano-naringin coated micronized lactose, which displayed outstanding in vivo pharmacokinetic performance and excellent dissolution properties (Ma et al., 2023).

4.2.3. Spray Freeze-Drying

SFD is an innovative technique which combines SD and classic freeze-drying. SFD is a continuous three-step method comprising atomisation, freezing, and drying. Initially, the drug solution is atomised; then, the resulting droplets are rapidly frozen using nitrogen as a refrigerant (Duong et al., 2021). The features of frozen granules may vary according to the set-up chosen for the first two steps, also referred to as the spray freezing (SF) stage. Sprayed droplets can be frozen in a chamber filled with cryogenic fluid in a vapour state (SF into vapour), liquid state (SF into liquid) or both (SF into vapour over liquid). The most used configuration is the last one, where the refrigeration of atomised particles starts in the vapour phase and ends in the liquid one (Adali et al., 2020). After SF, frozen particles are subsequently lyophilised, obtaining dry powders. Due to the low temperatures involved, SFD is particularly suitable for thermolabile substances (Duong et al., 2021). SFD allows for improved aerosolization properties compared to SD but involves higher costs and complexity (Pramanik et al., 2021).

SFD is an excellent method to formulate porous and low-density particles, owing to the sublimation of ice crystals during the drying step (Liao et al., 2019). In a study conducted by Parsian et al. (2014), inhalable porous MPs of budesonide were produced by SFD from a solution containing HPβCD and L-leu. The obtained particles exhibited good aerosolization properties regarding MMAD and FPF (Parsian et al., 2014). In another study, SFD was successfully applied to prepare inhalable dry powders of the antifungal agent voriconazole, employing mannitol as a bulking agent. Drug content and mannitol concentration were mainly responsible for the aerosolization and the structural integrity of the resulting particles, respectively (Liao et al., 2019). In a recent investigation, Liang et al. (2018) used SFD to prepare inhalable dry powders of siRNA, demonstrating that the MMAD of the siRNA particles could be reduced by increasing the gas flow rate in the atomizer. As an alternative to SD, SFD has also been suggested to produce inhalable dry powders of drug-loaded NPs (Ali and Lamprecht, 2014; Cheow et al., 2011; Yang et al., 2013). For instance, a suspension of the anti-SARS-CoV-2 agent niclosamide (nanocrystals) was spray freeze-dried with L-leu. Polysorbate 80 and 1,2-Dioctadecanoyl-*sn*-glycero-3-phophocholine were employed to stabilise the nanosuspension. In this study, the freezing temperature was varied between −80 °C and −40 °C, highlighting that freezing at −80 °C led to more open pores on the particle surface and better aerodynamics (Zhang et al., 2023). Additionally, SFD can be a viable method to produce inhalable biologics as recently surveyed by Farinha et al. (2023). For example, the pulmonary delivery of the monoclonal antibody adalimumab could be achieved by SFD in the presence of amino acids such as L-leu, phenylalanine, and arginine. By contrast, the addition of glycine decreased the aerosolization performance of adalimumab despite its stabilising action (Emami et al., 2019).

4.2.4. Thin film freezing

TFF induces the formation of particles by ultra-rapid freezing. During this process, a drug solution is frozen by contact with a cold solid surface and then freeze-dried to remove the solvent (Overhoff et al., 2009). The outcome of TFF is a porous, brittle matrix directly inserted into the DPI. The high cooling rate prevents crystallisation, and the formation of a nanostructured amorphous material is more likely to happen (Beinborn et al., 2012). After DPI shearing, the matrix is divided into inhalable MPs, i.e., nanostructured aggregates with geometric diameters higher than 10 μm and low density (Sahakijpijarn et al., 2020a). The application of TFF to produce inhalable dry powders has been widely overviewed by Pardeshi et al. (2022). In a recent study, TFF was tested to prepare inhalable dry powders of the antiviral remdesivir, used in COVID-19 treatments. It was demonstrated that this technology could be exploited to limit the need for excipients in the formulation, hence promoting the pulmonary delivery of remdesivir (Sahakijpijarn et al., 2020a). A similar result was found by Sahakijpijarn *et al.*, who produced tacrolimus dry powders for inhalation by TFF. Other than the potential minimisation of the request for excipients, they observed that drug loading of TFF particles could be increased up to 95 % without altering the aerosol properties (Sahakijpijarn et al., 2020b). In a following study performed by Jara et al. (2021), TFF was used to develop inhalable powders of niclosamide, whose poor solubility makes it difficult to administer in other ways. Such powders showed remarkably higher FPF than the analogous niclosamine particles produced by SD (Jara et al., 2021). The production of inhalable dry powders containing monoclonal antibodies was also pursued by TFF. These engineered powders possessed outstanding aerodynamic properties and the formulation containing lactose and L-leu exhibited the highest FPF (Hufnagel et al., 2022). TFF can constitute a valid alternative to SD and SFD to engineer

dry powders of pDNA which is sensitive to the atomisation stresses. Lower solid contents and plasmid loading showed better aerosol performance, being the highest FPF reached by combining 2.5 % (w/w) plasmid, a mannitol to L-leu ratio of about 7:3 (w/w), and a total solid content of 0.25 % (w/v) (Xu et al., 2023). In a recent study, inhalable dry powders of microRNA-laden extracellular vesicles were produced by TFF. The structural characteristics and morphology of the vesicles were preserved, and the integrity of miRNA was confirmed by qPCR. Moreover, the *in vitro* deposition profile revealed the suitability of these particles for inhalation purposes in the presence of sucrose, trehalose, or mannitol (AboulFotouh et al., 2024).

4.2.5. Surface modification of inhalable particles

To avoid fast absorption, immune recognition, biofouling, and escape pulmonary clearance, stealth materials are conventionally incorporated into the surface of respirable particles, forming a hydration layer over the drug molecule (Jain et al., 2020). HA, DPPC, and PEG have been identified as the most appropriate stealth materials for inhalable particles (El-Sherbiny et al., 2015; Jain et al., 2020).

The use of HA has been previously described; therefore, it is not further detailed. DPCC is a phospholipid composing about 55 %-60 % of lung surfactants (Meenach et al., 2013). A study conducted by Evora et al. (1998) demonstrated that coating PLGA microspheres with DPCC resulted in lower macrophage phagocytosis compared to non-coated particles. Several investigations report the advantages of adding PEG to the formulation of inhalable powders (Evora et al., 1998; Meenach et al., 2013; Patel et al., 2012; Tewes et al., 2011), including increased residence time, stability, and mucus-penetrating properties (Jain et al., 2020; Meenach et al., 2013). For instance, PEG-PLGA block copolymers can carry hydrophilic and ionised drugs, decreasing their uptake by alveolar macrophages compared to PLGA particles (Patel et al., 2012). In addition, binding peptides to PEG can increase their half-life, lower their immunogenicity, and avoid activity losses (Tewes et al., 2011). PEGylation of liposomes discourages their aggregation, improves uniformity, dispersibility, permeability and therapeutic effect towards bacterial biofilms, but lowers the retention time (Ibaraki et al., 2020). For example, De Leo et al. (2018) prepared PEG-surface-modified liposomes loaded with BDP, which exhibited enhanced mucus penetration than unmodified liposomes, suggesting their potential therapeutic effectiveness towards chronic respiratory diseases. In another study, drug release and absorption could be controlled by developing PEG-modified polylysine dendrimers, whose bioavailability increased with the length of PEG chains (Ryan et al., 2013).

In carrier-free formulations, the coating is also helpful in preventing cohesion among particles. The non-polar aliphatic amino acid L-leu has been widely investigated to reduce particle aggregation and protect spray-dried powders from moisture (Zillen et al., 2021). In most cases, the coating is performed by co-SD the drug and L-leu, which crystallises on the particle surface creating a hydrophobic layer and reducing particle interaction (Schoubben et al., 2019). In a study conducted by Raula et al. (2009), coating SS powders with L-leu enhanced particles' surface roughness reducing particle dispersion, and this effect was more intense as the size of L-leu crystals increased. This amino acid also demonstrated anti-adherent property in many applications such as in rifampicin-loaded PLGA MPs modified with L-leu, whose FPF increased with the L-leu concentration, reaching a maximum of 0.2 % w/v (Takeuchi et al., 2018). Since the co-SD process requires high costs and time to be performed, alternative coating techniques have been proposed. For example, Schoubben et al. (2019) produced budesonide MPs physically mixed with spray-dried-D-leu. In this study the enantiomeric form of the amino acid was used, resulting in powders with good *in vitro* aerosolization performances and suggesting physical mixing as a valid alternative to co-SD (Schoubben et al., 2019).

5. Conclusions and future perspectives

The interest in pulmonary delivery of therapeutics is constantly growing, as testified by a large amount of literature published on this topic in the last decades. This trend is justified by the significant advantages that inhalation of drugs offers compared to other administration routes, including huge surface area and low enzymatic action. Among the various formulations of therapeutic aerosols, respirable dry powders are the most convenient owing to their high stability. Such particles should respect some fundamental requirements in terms of size, shape, charge, and aerodynamic properties. Generally, particles with d_a of about 1–5 μm and low density are the best choice to achieve deep lung deposition.

As mentioned above, although therapeutic compounds are traditionally delivered through coarse lactose carriers, several issues are related to using this sugar in respirable dry powders. Therefore, drug-loaded MPs and NPs have been introduced as carrier-free formulations. The development of novel formulations was possible due to the widespread of modern production techniques as an alternative to traditional milling. Apart from improving SD and SFD, current research is focused on developing new platform technologies, including particle replication in nonwetting templates (PRINT®), inkjet printing, hot melt extrusion, and inhaled small particles easily respirable and emittable (iSPERSE™) technology (Mehta, 2019).

Despite being widely investigated, drug administration by inhalation is still affected by important limitations, which are mainly related to the natural defence mechanisms of human lungs, such as mucociliary clearance and macrophage-mediated clearance, and the incorrect use of the inhalation device. Moreover, due to their small size, inhalable particles are highly cohesive and with poor flowability. Many efforts have been made to overcome these barriers and develop engineered particles with appropriate sizes and surface properties. Since many factors influence the manufacture of respirable dry powders, one of the major aims of particle engineering is to change a single parameter without modifying the others (Scherließ et al., 2022). Particle engineering is expected to have a leading role in future research towards both carrier-based and carrier-free formulations. First, carrier engineering aims to balance drug adhesion and detachment, tailoring drug loading capacity, and customising surface properties (Scherließ et al., 2022). Regarding carrier-free formulations, the main goal is ensuring powder flowability, deposition, and targeting by modifying particle surface. Furthermore, achieving the successful treatment of pulmonary diseases through NP-based dry powders is a target since this therapeutic approach is still in the initial phase of clinical implementation (Pramanik et al., 2021).

Besides being employed for therapeutic purposes, pulmonary delivery could be used as a diagnostic tool for respiratory diseases (Chandel et al., 2019). Being small and non-invasive, inhalable NPs could be used to detect changes in airspace dimensions in lungs affected by emphysema (Strzempek et al., 2019). Specifically, magnetic particles hold the potential to be used as imaging tools for lung cancer diagnosis, especially when combined with magnetic resonance imaging (Saadat et al., 2020).

Whether applied as therapeutic agents or diagnosis tools, the administration of dry powders via inhalation could gain a primary role in the pharmaceutical industry's future. Continuous studies concerning the production and formulation of particles and lung biology are required to improve the efficacy of this drug delivery route.

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