Delivery strategies for sustained drug release in the lungs☆

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Abstract

Drug delivery to the lungs by inhalation offers a targeted drug therapy for respiratory diseases. However, the therapeutic efficacy of inhaled drugs is limited by their rapid clearance in the lungs. Carriers providing sustained drug release in the lungs can improve therapeutic outcomes of inhaled medicines because they can retain the drug load within the lungs and progressively release the drug locally at therapeutic levels. This review presents the different formulation strategies developed to control drug release in the lungs including microparticles and the wide array of nanomedicines. Large and porous microparticles offer excellent aerodynamic properties. Their large geometric size reduces their uptake by alveolar macrophages, making them a suitable carrier for sustained drug release in the lungs. Similarly, nanocarriers present significant potential for prolonged drug release in the lungs because they largely escape uptake by lung-surface macrophages and can remain in the pulmonary tissue for weeks. They can be embedded in large and porous microparticles in order to facilitate their delivery to the lungs. Conjugation of drugs to polymers as polyethylene glycol can be particularly beneficial to sustain the release of proteins in the lungs as it allows high protein loading. Drug conjugates can be readily delivered to respiratory airways by any current nebulizer device. Nonetheless, liposomes represent the formulation most advanced in clinical development. Liposomes can be prepared with lipids endogenous to the lungs and are particularly safe. Their composition can be adjusted to modulate drug release and they can encapsulate both hydrophilic and lipophilic compounds with high drug loading.

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☆ This review is part of the Advanced Drug Delivery Reviews theme issue on “Improving the efficacy of inhaled drugs for severe lung diseases: emerging pulmonary delivery strategies”.
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1. Introduction

Drug delivery to the lungs by inhalation offers a targeted drug therapy for respiratory diseases. The local route of drug administration allows one order of magnitude-lower drug doses to be delivered, compared to systemic administration by the oral route or by injection. The low dosing locally reduces systemic exposure to the drug, and thereby systemic side effects, and increases drug therapeutic index. In addition, portable inhalers make this route of drug administration convenient for the patient. Inhalation aerosols are developed for drug administration to the systemic circulation as well. The large absorptive surface area of the alveoli, the very thin diffusion path from the airspaces into the blood and the elevated blood flow make the lung a port of entry to the systemic circulation. Drug molecules are absorbed more efficiently from the lung than from any other non-invasive routes of drug administration. As a result, a continuously increasing number of inhaled drugs are becoming available on the market to treat various diseases [1].

However, the therapeutic efficacy of inhaled drugs is limited by their rapid clearance in the lungs [2]. Small solutes delivered to the lungs quickly diffuse across lung epithelia and penetrate the bloodstream within minutes. Peptides are rapidly transported to the systemic circulation as well, but are significantly metabolized locally [3,4]. Although macromolecules can be absorbed into the systemic circulation over several hours, they can be rapidly taken up by alveolar macrophages, they can be removed by the mucociliary escalator and they can be metabolized locally as well. For instance, recombinant human deoxyribonuclease I is a 37 kDa glycoprotein which cleaves the DNA in respiratory secretions of cystic fibrosis patients and thus, lowers their viscosity [5]. This glycoprotein is the mucolytic agent most widely used in the symptomatic treatment of cystic fibrosis. However, it is rapidly cleared from the human lungs: when the daily dose of 2.5 mg is inhaled, a concentration of 3 μg/ml is measured in sputum immediately after inhalation and it is reduced to 0.6 μg/ml after 2 h. Therefore, its once to twice daily administration provides limited therapeutic coverage to the patients. The short residence time of drugs within the lungs can also imply frequent dosing and this can jeopardize patient compliance. It is for instance recommended to inhale corticosteroids twice daily and short-acting β2-agonists up to 4-times daily.

Carriers providing sustained drug release in the lungs could improve therapeutic outcomes of inhaled medicines. Their objectives are to retain the drug load within the lungs for an extended period of time and to progressively release the drug locally at therapeutic levels. Sustained therapeutic drug concentrations should improve local therapeutic efficacy and further decrease systemic side effects as the biodistribution throughout the systemic circulation is minimized. In addition, a sustained-release inhaled formulation could avoid peaks in local drug concentrations that could be toxic to the pulmonary tissue. This is particularly relevant for chemotherapeutic agents. Sustained drug release in the lungs could also benefit to systemically-acting drugs by controlling the rate of drug release and transport into the systemic circulation.

Although sustained-drug release in the lungs presents high potential to improve the therapeutic efficacy and safety of inhaled drugs, there is not yet any sustained-release formulation available in the market. Only a few sustained-release formulations are in clinical development and all are in the form of liposomes (Table 1). In recent years, tremendous efforts in the area have focused on the development of new inhaler devices and new formulations with the goal to increase pulmonary deposition and its reproducibility. However, a further degree of sophistication in inhalation aerosols could be reached through the development of controlled-release formulations.

This review will present the main parameters involved in the local availability of inhaled compounds as well as the different strategies developed to control drug release in the lungs including microparticles, insolubilization and the wide array of nanomedicines.

2. Factors affecting the local availability of inhaled compounds

Several factors affect the local availability of drugs following delivery to the lungs. First, the ability of an aerosol particle to settle in one or another region of the respiratory tract depends on its aerodynamic diameter. Second, the pulmonary fate and rate of clearance of a drug from the lungs are determined by its physico-chemical and biological properties. Third, if the drug formulation is not merely the drug molecule but in-solubilized and the wide array of nanomedicines.

The site of deposition of an aerosol particle within the lungs depends on its aerodynamic diameter ($d_{50}$) and on the breathing pattern of the patient. The aerodynamic diameter of a particle is equivalent to the diameter of a unit density ($\rho_0$) sphere that has the same terminal velocity.
in still air as the particle:

\[ d_{\text{ge}} = d \sqrt{\frac{\rho_f}{\rho \chi}} \]

where \( d \) is the geometric diameter of the particle, \( \rho \) is the particle density and \( \chi \) is the particle dynamic shape factor denoting deviation of shape from sphericity [13].

Filtering of large particles (\( d_{\text{ge}} > 5 \mu \text{m} \)) occurs in the upper airways (mouth, trachea and main bronchi) by inertial impaction. 1 to 5 \( \mu \text{m} d_{\text{ge}} \) particles deposit by gravitational settling in the central and distal tracts. Particles with \( d_{\text{ge}} \) between 0.1 and 1 \( \mu \text{m} \) are mostly exhaled. Slow inhalation is generally preferred to minimize inertial impaction in upper airways and to increase penetration into the lungs of large particles, whereas small particles are much less sensitive to fast/slow inhalation maneuvers [20]. A breath hold gives time to particles that have penetrated deep into the lungs to sediment on airway surfaces. Ultrafine (<100 nm) particles efficiently deposit by random Brownian motion in the respiratory tract: particles <100 nm reach the alveolar region while particles <10 nm already deposit in the tracheo-bronchial region due to their high diffusion coefficients [15].

As seen from the equation defining \( d_{\text{ge}} \), other parameters than the geometric diameter of the particle are involved in lung deposition. Particle density is an important parameter that can adjust \( d_{\text{ge}} \). Accordingly, large (>5 \( \mu \text{m} \)) particles can be successfully inhaled deep into the lungs as far as their density is low [16]. Particle shape can be designed to target different regions of the respiratory tract. For instance, fibers have an aerodynamic diameter few times smaller than their length, which permits deep lung deposition [17]. Unfortunately, a higher lung toxicity has been reported for elongated particles, compared to spherical particles [18]. Charged particles deposit more readily in the lungs than neutral particles [19]. Moisture absorption may increase particle size, which may shift pulmonary deposition upwards [20].

### 2.2. Mechanisms of drug absorption from the lungs

It has been reported that lung epithelia are highly permeable to a wide range of drug types. Lipophilic and non-ionized compounds are transported across respiratory epithelia into the bloodstream more rapidly and in larger amounts than hydrophilic compounds. In general, small hydrophilic compounds (\( \log P < 0 \)) have a mean half-life to absorption of around 1 h, whereas lipophilic small molecules (\( \log P > 0 \)) are absorbed in approximately 1 min (Fig. 1) [21]. Furthermore, the rate of macromolecule transport from the airway lumen into the systemic circulation is inversely related to molecular weight. Macromolecules with a molecular weight above 40 kDa are absorbed over several hours, in contrast to peptides or smaller proteins which reach the bloodstream within a few minutes following inhalation [22]. Peptides and small proteins can be subject to local proteolysis and large macromolecules can be cleared by alveolar macrophages [22,23]. The prolonged presence of macromolecules within the lungs can be utilized to sustain drug presence within the respiratory tract by drug conjugation to macromolecules [21].

The mechanisms of transport across respiratory epithelia depend on the physico-chemical and biological properties of the drug. Lipophilic drugs are mainly absorbed by passive diffusion through the cells whereas hydrophilic drugs are absorbed by diffusion through tight junctions. Some compounds have been shown to use drug transporters as active mechanism of absorption. High levels of drug transporters are expressed in the lungs. Many efflux transporters expressed in the intestine, liver, kidney or brain are also present in the lungs [24]. For instance, iratropium bromide, an anticholinergic drug, is a substrate of the organic cation transporter Octn2 and is actively absorbed by this transporter within the tracheal tissue [25]. Moreover, P-glycoprotein-mediated efflux has been shown to prevent pulmonary absorption of rhodamine 123 and loperamide from the lung airways to the perifusate in an isolated perfused rat lung model [26]. Macromolecules can be transported by receptor-mediated transcytosis, by para-cellular diffusion (<40 kDa) and/or by non-specific pinocytosis (<40 kDa) [27].

The amount of drug absorbed from the lungs into the bloodstream is generally higher following deposition of particle aerosols in the lung periphery than after particle deposition in the central region of the lungs [28]. However, drug transport can occur across both the airway pseudostratified columnar epithelium and the thin alveolar epithelium [29]. Respiratory epithelia present a tighter barrier to the transport of compounds towards the bloodstream than does the capillary endothelium [30].

### 2.3. Clearance of carrier particles in the lungs

Two major mechanisms are involved in the clearance of particles in the lungs. Although these mechanisms protect the airways from disastrous exposure to foreign materials, they also represent barriers to pulmonary drug delivery. Phagocytosis by lung-surface macrophages is the main clearance mechanism of microparticles in the lungs. However, it is inefficient to clear particles smaller than 200 nm. Semmler-Behnke et al. compared macrophages uptake of 18 nm iridium-192-radiolabeled particles and 2.1 \( \mu \text{m} \) polystyrene particles after inhalation in rats. Twenty percent of the recovered radioactivity was associated with alveolar macrophages over 3 weeks after nanoparticle delivery whereas 80% of the recovered microparticles were retained within the alveolar macrophages over the same period [31]. Geiser et al. studied the role of lung surface macroparticles in the clearance of inhaled 20 nm titanium dioxide particles from rat lungs. They found an uptake of approximately 0.1% of the inhaled dose by lung surface macroparticles over 24 h [32].

The mucociliary escalator eliminates particles and solutes deposited in the airways [2,33]. Mucociliary clearance velocities in respiratory airways decrease with airway diameter [34]. The mucus velocity measured in the human trachea is 5.5 mm/min and it is already decreased to 2.4 mm/min in the main bronchi [35]. Möller et al. targeted 100 nm technetium 99 m-labeled carbon particles to the airways or lung periphery using a bolus inhalation technique in healthy volunteers. A respiratory aerosol probe allowed aerosol inhalation under controlled breathing conditions and the application of an aerosol bolus to a predefined lung depth. Lung retention of the nanoparticles targeted to the airways and to the lung periphery was 75% and 96%, respectively, after 24 h [36]. Mucociliary clearance was the likely cause of the decreased retention following nanoparticle administration to the airways. The composition and clearance of respiratory mucus are often altered in patients suffering from respiratory diseases and this may affect the fate of inhaled drug carriers. It has been reported that the thickness of the mucus layer increases from 2 to 30 \( \mu \text{m} \) in healthy lungs to more than 250 \( \mu \text{m} \) in cases of cystic fibrosis and other obstructive airway diseases [37].

![Fig. 1. Drug absorption from the lungs is dependent on drug lipophilicity. The mean half-life to absorption is the time needed for half of the molecules deposited in the lungs to disappear from the tissue. Log P represents the octanol/water partition coefficient. Squares represent lipid insoluble molecules, triangles, lipid soluble molecules and inverted triangles, molecules with active uptake [21].](image-url)
3. Strategies for controlling drug release in the lungs

Several formulation strategies have been developed to sustain drug release in the lungs and particulate carriers have been the most investigated approach. They allow the release of the drug in a controlled manner at a therapeutically optimal rate. These systems present several advantages over the soluble drugs namely i) an increased drug residence time in the lungs, ii) a protection of the therapeutic agent against local degradation, iii) the possibility to target drugs to specific cells, and iv) a stability against forces generated during aerosolization.

The next paragraph will discuss the different mechanisms involved in drug release from carriers. Then, the various delivery strategies that have been developed to control drug release in the lungs will be presented.

3.1. Drug release mechanisms from carrier particles

In order to become active locally, the drug should first be released from its carrier. The drug release mechanism from any carrier particle primarily involves drug diffusion through the carrier material. Additional mechanisms depend on the drug carrier considered [38].

Drug release from polymeric carriers involves several mechanisms. Depending on the polymeric structure, the drug might be released by diffusion through the polymeric matrix or through pores present in the carrier. Erosion of the carrier surface or bulk erosion can also lead to drug release [33,39]. The high surface area to volume ratio of polymeric carriers contributes to a rapid drug release from the matrix which is called a “burst”. This phenomenon can be reduced by drug complexation with various agents before encapsulation as well as by adding lipid aid excipients to the formulation [40]. Complexing agents or lipid aid excipients interact with the encapsulated compound and these interactions increase encapsulation efficiency as they decrease the burst [41].

Drug release from micelles or dendrimers involves drug diffusion and micelle or dendrimer break-up. Drug release rate depends on the rate of drug diffusion within the delivery system, the drug partition coefficient, the stability of the micelles and the rate of polymer degradation [38].

The most important mechanism of drug release from liposomes or solid lipid nanoparticles is drug diffusion through the lipid bilayers or lipid matrix. Drug release may be modulated by the lipid composition, the glass transition temperature of the phospholipids and the multi-lamellarity of the liposomes [38]. For instance, the presence of rigid phospholipids or cholesterol decreases liposome membrane fluidity, which leads to a less permeable membrane [42].

In addition, drug release from a carrier particle may be triggered by different stimuli such as temperature, ultrasound and pH [43]. However, except for temperature, these mechanisms have not been reported yet in pulmonary drug delivery research. Lipid microparticles loaded with budesonide and superparamagnetic iron oxide nanoparticles (SPIONs) presented both thermo-sensitive and magnetic characteristics in vitro suitable for controlled and targeted delivery, respectively. The thermo-sensitive lipid system, based on the solid lipid glyceryl behenate, allowed slow drug release at body temperature but fast release at hyperthermic temperature [44].

3.2. Poorly soluble drugs and coprecipitates

The intrinsic poor solubility of some drugs such as corticosteroids, sex hormones and antifungals contributes to sustain drug release in the lungs [45]. For instance, fluticasone presents a lower solubility than budesonide and this lower solubility results in a slower absorption rate from the lungs following inhalation [46]. Estradiol formulated as large and porous particles provided sustained systemic estradiol concentrations for 5 days following inhalation in rats [47].

Another approach to modulate drug release consists of creating an insoluble complex with the drug. Insulin forms an insoluble complex with protamine and/or zinc chloride. Insoluble insulin was delivered as large porous particles to the lungs of rats and provided sustained plasma insulin levels for half a day, similarly as subcutaneous injection of the insoluble complex did [48]. Still another approach involves the agglomeration of nanoparticles. Nanometer-sized drug particles can be assembled in micron-sized clusters with the desired aerodynamic diameter. For instance, nanoparticle agglomerates of fluticasone exhibited slower dissolution rate than single fluticasone nanoparticles [49].

3.3. Microparticles

Conventional inhaled particles with geometric sizes between 1 and 3 μm and mass density near 1 g/cm³ are prone to particle aggregation in the dry powder inhaler and to rapid clearance by macrophages in the lung lumen [16]. The preparation of particles with large geometric size (>5 μm) and low mass density (<0.4 g/cm³) may alleviate these limitations [16]. The large geometric size of large porous particles (LPPs) leads to ease of particle dispersion. Their small aerodynamic size, resulting from their low particle density, allows large porous particles to effectively escape impaction in upper airways and penetrate deep into the lungs. Apart from excellent flow properties, large porous particles may more easily escape phagocytosis by alveolar macrophages due to large geometric sizes and may therefore render the pulmonary administration of sustained-release formulations attainable because of diminished clearance of drug particles [16,50,51].

Poly(lactic-co-glycolic acid) (PLGA) represents the most investigated polymer for the preparation of sustained-release microspheres for pulmonary administration [41,52]. PLGA is biocompatible and biodegradable. It is an excipient already found in several microsphere products for injection in the market. PLGA does not present toxicity toward several human airway cell lines in vitro and indication of safety following pulmonary delivery in vivo has been provided in the mice [41,53]. However, the FDA has not yet approved PLGA as excipient for inhalation. The degradation rate of PLGA can vary from 3 weeks to over a year, depending on the polymer molecular weight and lactic acid to glycolic acid mass ratio. Polymeric microparticles can encapsulate both hydrophilic or lipophilic compounds, according to particle formulation method.

In their initial work, Edwards et al. encapsulated insulin into large porous PLGA particles prepared by the double-emulsion solvent evaporation technique and obtained sustained serum insulin levels for 4 days following pulmonary delivery in rats. For comparison, small non-porous insulin particles had high serum insulin levels for only 4 h [16]. Several other research groups have then prepared large porous PLGA particles (Fig. 2) [54,55]. Oh et al. prepared budesonide-loaded large porous PLGA microparticles using the double-emulsion solvent evaporation method and ammonium bicarbonate as porogen. Budesonide was released from the particles in a sustained-manner for 24 h in vitro and the particles better reduced inflammation in a murine model of ovalbumin-induced lung inflammation than the free drug or budesonide-loaded non-porous particles [56].

However, microparticles prepared with PLGA present inherent limitations. These include the limited drug loading, the burst release of the drug, the risk of polymer accumulation within the lungs and the core environment unfavorable to drug stability. Polymeric particles classically present a maximum drug loading of 10% [55,57], which means that they can only be applied to relatively low-dose drugs. In fact, mass loads that can be inhaled in one inhalation are limited to 25 or 50 mg, depending on the dry powder inhaler device [58,59]. The initial burst release of the drug originates from drug diffusion from the particle surface and the subsequent sustained drug release from the hydrolytic degradation of the polymer mass. Particle porosity increases the particle surface in immediate contact with the release medium and increases the initial
burst. More than 50% of the drug load can be released within a few hours and this burst needs to be minimized through optimization of the formulation [55,60]. Polymer accumulation within the lungs might arise as drug release proceeds over shorter periods than complete degradation of the polymer. Bulk degradation of PLGA creates an acidic core which can damage pH sensitive drugs such as peptides or proteins [61].

Osmotic agents have been used to create porosity in PLGA microparticles. For instance, bovine serum albumin dissolved in the internal water phase induced osmotic flow between the internal and external water phases during the double emulsion process, resulting in the fabrication of microspheres with controllable, uniform porous structures. In addition, bovine serum albumin reduced the initial burst of encapsulated oppositely charged VEGF from the porous microspheres and sustained VEGF release for 2 weeks [62]. The incorporation of polyethyleneimine in PLGA microparticles also resulted in highly porous microparticles, owing to an osmotic gradient between the particle core and the external aqueous phase [63].

Hydroxypropyl-β-cyclodextrin has been used as excipient in the preparation of large porous PLGA particles in order to slow insulin release from the microparticles as well as to optimize the aerodynamic properties of the dry powder [60,64]. The formation of insulin/hydroxypropyl-β-cyclodextrin complexes within the microspheres decreased protein diffusivity in the polymer matrix and thereby decreased insulin burst and slowed overall insulin release. In addition, the incorporation of hydroxypropyl-β-cyclodextrin in the internal phase of the microspheres enabled the formation of porous microspheres due to osmotic flow towards the internal phase [60]. Large porous particles of PLGA/hydroxypropyl-β-cyclodextrin/insulin were able to reach the
deep lungs and to release insulin in the alveolar tissue. Insulin was then rapidly absorbed systemically in a bioactive form allowing a rapid onset of hypoglycaemic effect. The developed large porous particles were tested in hyperglycaemic rats and exerted a significant and longer hypoglycaemic effect as compared with an insulin solution.

Other materials than PLGA have been used to prepare microparticles for prolonged drug release in the lungs. These include other polymers as alginate, chitosan or poly(glycolic adipate-co-ε-o-ε-pentadecalactone), a biodegradable polyester, as well as lipids as dipalmitoylphosphatidylcholine, tristearin, compitol and glyceryl behenate [50,65–70]. Inhalation of large porous particles made of albuterol, lactose, albumin and a high percentage of dipalmitoylphosphatidylcholine provided protection against carbachol-induced bronchoconstriction for 16 h in guinea pigs [50]. Budesonide release from solid lipid microparticles of compitol was significantly longer in vitro as compared to the release from crystalline or amorphous powder of budesonide. The slow release can be explained by the complex diffusion process in solid lipid microparticles [67].

3.4. Nanomedicines

Nanomedicines are a large field of nanosized systems that can be divided in two categories: i) nanocarriers which include polymeric nanoparticles, micelles, liposomes, and solid lipid nanoparticles and ii) drug conjugates which include dendrimers and polymer conjugates (Fig. 3). The sizes of these systems range from a few nanometers to 1 μm [37]. Nanomedicines are of the same size as biological entities and can readily interact with biomolecules on both the cell surface and within the cell [2]. The combination of this attractive feature with interesting drug delivery properties has led to the development of a wide array of nanomedicines which will be reviewed below.

3.4.1. Polymeric nanoparticles

Nanoparticles are particularly interesting carriers for sustaining drug release within the lungs because they largely escape uptake by lung-surface macrophages and can remain in the pulmonary tissue for weeks [2]. Polymers used to formulate nanoparticles are the same as those used to formulate microparticles and principally include PLGA but also poly-ε-caprolactone, chitosan and alginate [71]. However, one needs to keep in mind that the same limitations arise for PLGA nanoparticles as for PLGA microparticles.

Nanoadsols require enormous energy for their creation and medical dry powder inhalers are not capable to disperse nanosized dry powder particles. Therefore, nanoparticle drug formulations are most often delivered to the respiratory tract by nebulization of colloidal suspensions [72]. The incorporation of nanoparticles within large porous dry powder particles is another way to administer nanoparticles to respiratory airways [73]. Once deposited in the lungs, the microparticle carriers dissociate to yield the nanoparticles with their preserved release properties and inherent pulmonary persistence.

Ungaro et al. prepared tobramycin PLGA nanoparticles by an emulsion/solvent diffusion technique and embedded the nanoparticles in an inert microcarrier made of lactose. Different helper polymers were added to PLGA in order to optimize the nanoparticle size, surface, release properties and encapsulation efficiency. Poly(vinyl alcohol) and chitosan allowed to optimize the size and to modulate the surface properties of the PLGA nanoparticles, whereas the use of alginate allowed efficient tobramycin entrapment within the nanoparticles and its release up to 1 month in vitro. Spray-drying of the nanoparticles with lactose led to dry powders presenting good aerolization properties [74].

Chitosan coating has been shown to increase the residence time of PLGA nanoparticles in the lungs, probably due to its mucoadhesive properties [75]. The antiabetic drug, palmitic acid-conjugated exendin–4, was encapsulated in chitosan-coated PLGA nanoparticles. The drug was released over 3 days in vitro and produced hypoglycemia over 4 days in mice. However, the use of chitosan for pulmonary delivery is not recommended because chitosan opens tight junctions of epithelia and may cause pulmonary edema [76].

Coating nanoparticles with inert biocompatible polymers such as polyethylene glycol (PEG) represents the most popular strategy of surface functionalization. Coating particles with PEG creates a hydrophilic and neutral shell that minimizes adhesive interactions of the nanoparticle core with mucus. Particles coated with 2 or 5 kDa PEG have been shown to present rapid mucus-penetrating properties whereas particles coated with 10 kDa PEG were trapped within the mucus in vitro [77]. In addition, PEGylation of nanoparticle surface might further safeguard them against clearance by lung surface macrophages [78]. However, the impact of PEGylation the particle surface on the residence time of carrier particles within the lungs has not been explored yet.

3.4.2. Micelles

Micelles are colloidal particles formed of amphiphilic block copolymers or surface active agents [79]. In general, their size ranges from 5 to 100 nm. At low concentrations in aqueous medium, the amphiphiles exist as monomers. Above the critical micelle concentration, the monomers self-assemble to form micelles characterized by a hydrophobic core and a hydrophilic shell. Micelles are used as carriers of poorly soluble compounds where solubilization occurs in the micelle core. The loading efficacy of micelles towards hydrophobic drugs ranges between 5 and 25% by weight. In addition, micelles can avoid alveolar macrophages uptake due to their small size and bulky hydrophilic outer shell and they can prolong drug release [22].

A variety of amphiphilic materials can be used to form polymeric micelles. The hydrophilic part of the micelles usually comprises PEG with a molecular weight from 1 to 15 kDa. The hydrophobic part may be formed of phospholipids or hydrophobic polymers. Lipid moieties as hydrophobic blocks provide additional micelles stability compared to conventional polymeric micelles: the two fatty acyls of phospholipids increase hydrophobic interactions between chains in the core of the micelles [79]. Interestingly, phospholipids that are endogenous to the lung can be selected in order to create a carrier suitable for pulmonary drug delivery. This is the case of diacylphosphatidylethanolamine, a constituent of the lung surfactant, used to form PEG–diacylphosphatidylethanolamine micelles of size from 5 to 35 nm [79].

Polyethylene glycol-di-stearylphosphatidylethanolamine micelles were investigated as carrier for pulmonary delivery of beclomethasone dipropionate. The micelles increased the solubility of beclomethasone dipropionate by 1300 times and prolonged its release in vitro. The drug release from the micelles occurred over 3 days whereas it occurred over a few hours from plain beclomethasone dipropionate. A significant reduction in the inflammatory cell counts in bronchoalveolar lavage fluid samples resulted from the intratracheal administration of the beclomethasone dipropionate-loaded micelles, compared to free beclomethasone dipropionate, in an ovalbumin-induced inflammation rat model [80].

Gill et al. loaded PEG–di-tearoylphosphatidylethanolamine micelles with paclitaxel and studied their fate following pulmonary delivery in rats. The micelles retarded paclitaxel release over 8 h in vitro and better retained paclitaxel within the lungs in vivo than paclitaxel formulated in cremophor and ethanol [81].

Todoroff et al. showed that Poloxamer 407 micelles increased the pulmonary residence time of the model antigen ovalbumin in mice following their co-administration. The combination of P407 with the vaccine adjuvant CpG oligonucleotide induced the highest Th-1 and Th-17 immune responses to M. tuberculosis antigen 85A compared to the adjuvants alone. The authors hypothesized that the increased viscosity of the P407 micelles allowed the copolymer solution to act as an antigen depot [82].

3.4.3. Liposomes

Liposomes are the only sustained-release strategy for pulmonary delivery that has reached clinical development. Liposomes are artificial
vesicles formed of lipid bilayers and can encapsulate both hydrophilic and hydrophobic drugs. The lipid bilayers are basically composed of phospholipids and cholesterol. The polar head and hydrophobic tail of phospholipids allow the formation of lipid bilayers, whereas cholesterol rigidifies and stabilizes the liposome membrane. The lipid composition in liposomes may modulate drug release. For instance, rigid phospholipids or cholesterol decrease membrane fluidity and lead to a less permeable membrane for drugs to diffuse through [42]. Liposomes are a particularly suitable drug delivery vehicle for pulmonary administration because liposomes are safe and well tolerated by the lungs. In particular, they can be prepared by using phospholipids and neutral lipids endogenous to the lungs such as dipalmitoylphosphatidylcholine, the major phospholipid of lung surfactant, and cholesterol [83]. In addition, the versatility in composition can provide various fates of liposomes in vivo [84].

Liposomes can be classified in terms of size and number of lipid bilayers. Multilamellar vesicles (MLVs) are made of multiple lipid bilayers and present sizes from a few hundred nanometers to several micrometers. They have a moderate aqueous volume to lipid ratio (1–4 μl/μmol lipid) and better encapsulate lipophilic compounds than hydrophilic ones due to possible incorporation within each lipid bilayer. Large unilamellar vesicles (LUVs) and small unilamellar vesicles (SUVs) have a single lipid bilayer with an aqueous core suitable for encapsulating hydrophilic compounds. LUVs are larger than 100 nm with an aqueous volume to lipid ratio of 7 μl/μmol lipid. SUVs are smaller than 100 nm and present an aqueous volume to lipid ratio of 0.2–1.5 μl/μmol lipid [83,84]. Drug release rate is faster from unilamellar liposomes than from multilamellar ones because the drug needs to cross only one bilayer [38].

Many different drugs have been encapsulated in liposomes for sustaining their release in the lungs. These include antibiotics [85,86], bronchodilators [87,88], immunosuppressants [89,90], anticancer drugs [91], sex hormones [92,93], peptides [94], proteins [95] and oligonucleotides [96].

Liposomes can be delivered to the human lungs by nebulization of a liposome suspension or as a dry powder [83,97]. However, nebulization can cause structural disruption of liposomes, which can result in the release of the encapsulated drug [98]. Dry powders of liposomes can avoid stability issues seen with nebulized solutions. They can be prepared by spray-drying, spray-freeze-drying and freeze-drying followed by micronization [98–100].

Beck-Broichsitter et al. prepared liposomes with different phospholipids and cholesterol and adjusted their phase transition temperatures below and above body temperature [42]. The transition temperature from the gel to the liquid phase determines whether the liposome membrane will be rigid or fluid at body temperature. The amount of carboxyfluorescein released from the carboxyfluorescein-loaded liposomes correlated well with their membrane fluidity and an increase in gel-to-liquid phase transition temperature resulted in an extended dye release profile. Nebulization with a vibrating-mesh nebulizer decreased the amount of encapsulated dye by 20%.

Antibiotics have been successfully encapsulated in liposomes and some liposomal antibiotics are in different stages of clinical development. For instance, Arikace®, a liposomal amikacin formulation is in phase III clinical studies for the treatment of *Pseudomonas aeruginosa* infections in patients with cystic fibrosis [101–103] and in phase II clinical studies for the treatment of nontuberculous mycobacterial lung disease [104, 105]. The liposomes are made with dipalmitoylphosphatidylcholine and cholesterol in a 2:1 weight ratio [106]. This composition confers a high gel-to-liquid phase transition temperature and therefore a high degree of membrane stability and reduced amikacin leakage. Amikacin is entrapped efficiently in the aqueous core of the 250–300 nm liposomes and the overall lipid-to-drug weight ratio was 0.7:1. Liposomal amikacin provided slow sustained amikacin release in normal uninfected rat lungs (Fig. 4). Free amikacin does not easily diffuse in mucus due to electrostatic interactions with mucin fibers. However, liposomal amikacin penetrates in sputum and biofilm and attains access and close proximity to bacteria. In addition, drug release is mediated by virulence factors (the rhamnolipids) produced by biofilm-localized *P. aeruginosa*. These properties make Arikace® a therapeutic with reduced dosing frequency (once daily) and greater efficacy in man compared to free aminoglycoside therapy (i.e., twice-daily tobramycin) [104,107].

A dual release liposomal ciprofloxacin formulation (Pulmaquin™) is in phase III clinical studies by inhalation [108]. This formulation has been developed in order to optimize drug delivery in the management of chronic lung infections with *P. aeruginosa* in patients with non-cystic fibrosis bronchiectasis. Although inhaled tobramycin presents microbiological efficacy in non-cystic fibrosis bronchiectasis, it also increases respiratory adverse events and is poorly tolerated by the lungs. Pulmaquin™ is composed of a mixture of free and liposomal ciprofloxacin which provides an immediate release (free drug) followed by a sustained release over 24 h (liposomal ciprofloxacin). In addition to providing sustained-release, Pulmaquin™ improves tolerability by minimizing the amount of free irritant antibiotic in direct contact with the airway [7,108].

### 3.4.4. Solid lipid nanoparticles

Solid lipid nanoparticles are stable colloidal carriers made of lipids solid at both room and body temperatures [109]. They are formed of a solid lipid core stabilized by a surfactant and containing the drug dissolved or dispersed. The rigidity of the lipid core is an important parameter which determines the rate of drug release. Despite the diversity of compounds involved in the preparation of solid lipid nanoparticles, they are well tolerated by the lungs and are considered carriers of low toxicity [110,111]. Compared to liposomes, solid lipid nanoparticles present the advantage of being more stable physically (e.g., during nebulization) [109].

Solid lipid nanoparticles have been shown to sustain the release of different drugs in the lungs. Li et al. prepared microparticles containing thymopentin-loaded solid lipid nanoparticles for pulmonary delivery. The microparticles showed similar sustained release of the systemically acting peptide as free solid lipid nanoparticles in vitro. Following delivery to the lungs in rats, the microparticles resulted in sustained plasma concentrations of thymopentin over 48 h, whereas the unencapsulated peptide was cleared from plasma within 1 h [112].

### 3.4.5. Dendrimers

Dendrimers are three-dimensional structures of hyperbranched homopolymers and form a tree structure. Dendrimers are composed of four main regions: an initiator core, interior layers or generations made of repeated units and attached to the core, terminal surface groups and spaces in which the drug may be entrapped. Dendrimers are also called “unimolecular micelles” because they are formed of an apolar core and a polar shell [38]. Dendrimers can be used as drug...
carrier through different mechanisms such as encapsulation within dendrimeric cavities or binding to the dendrimer surface by electrostatic interactions or covalent linkage [113]. Dendrimers increase drug half-life, can improve drug solubility and can protect biological tissues from drug toxicity. In addition, the surface of the dendrimers can be modified by attaching antibodies and ligands and make dendrimers more specific to certain tissues or organs [114].

A few studies showed that dendrimers can prolong drug retention within the lungs. Inapagolla et al. conjugated methylprednisolone to a polyamidoamine dendrimer, yielding 12 methyprednisolone molecules per dendrimer molecule. The dendrimer was retained in the lungs for 7 days and improved methylprednisolone efficacy in reducing allergen-induced lung inflammation in mice [115]. Ryan et al. showed that increasing the size of dendrimers from 11 kDa to 78 kDa led to slower absorption from the lungs into the bloodstream and to a more prolonged retention in the lung tissue and airspaces. The largest dendrimers were retained in the lungs for up to 7 days, providing potential for controlled drug delivery to the respiratory tissue or blood [116]. Kaminskas et al. conjugated a 56 kDa PEGylated polylysine dendrimer to doxorubicin to promote the controlled and prolonged exposure of lung cancer cells to the cytotoxic drug. The dendrimer sustained doxorubicin presence in the lungs for 7 days, whereas the free drug was rapidly cleared by absorption to the bloodstream. Twice-weekly intratracheal instillation of the doxorubicin dendrimer led to almost complete regression of lung tumor burdens, whereas intratracheal instillation of the free drug led to extensive lung-related toxicity and death within several days of a single dose [117].

3.4.6. PEGylation

PEGylation is a common process used to improve the therapeutic value of a medicine by prolonging its body residence time. It involves the attachment of one or more PEG chains on the entity and can be applied to proteins, peptides, particles, small molecules and cells [118]. Several PEGylated proteins are approved for clinical use by injection. Polyethylene glycol glycol is a neutral linear polymer with a wide range of molecular weights. The repeated ethylene moiety along the PEG chain is responsible for hydrophobicity, whereas oxygen confers strong interactions with water [119]. Consequently, PEG is soluble in both aqueous and organic media. PEG has two important properties, the high flexibility and the high hydration of its backbone. The carbon-carbon and carbon-oxygen bonds offer extended flexibility to the whole polymer, leading to a large exclusion volume of approaching molecules. Between two and three water molecules are bound per repeat monomer unit [120]. Consequently, the polymer has a hydrodynamic volume five to ten times higher than that of a globular protein of the equivalent molecular weight [121]. The large flexibility and hydration of the polymer create steric hindrance [122]. Steric hindrance excludes proteases, antibodies and opsonins from the vicinity of the medicine, thereby decreasing degradation of proteins and phagocytosis of particles. In addition, the increased hydrodynamic volume of the PEGylated compound decreases its clearance by the kidney and contributes to the prolonged in vivo half-life of the conjugated moiety. PEG is safe, non-biodegradable and approved by the FDA for use by various routes including the inhalation route in the case of small PEGs [123]. Its safety is assured by its established usage in foods, cosmetics and drugs [124].

PEGylated peptides and proteins have been delivered to the lungs with different purposes. PEGylated rhG-CSF and PEGylated insulin were delivered to the lungs in rats and/or dogs in order to test whether PEGylated proteins could be absorbed systemically from the lungs [125–127]. Once in the bloodstream, PEGylated proteins would provide sustained plasma concentrations of the protein as they would have been injected. PEGylated rhG-CSF and PEGylated insulin were both absorbed from the lungs. However, the extent of systemic absorption decreased following PEGylation on multi-protein sites and using large PEGs (5–12 kDa PEGs). PEGylation has also been used to protect peptides from local proteolysis in the lungs and thereby increase the systemic absorption of the intact molecule following pulmonary delivery [128,129].

Site-specific PEGylation of calcitonin by 1, 2 or 5 kDa linear PEG increased the peptide stability in rat lung homogenates by up to 3 orders of magnitude. As a result, systemic bioavailabilities of the conjugates increased up to 8-fold following pulmonary delivery. In addition, the conjugates were much less rapidly removed from the systemic circulation. A recent study demonstrated that the conjugation of antibody fragments to a large PEG greatly prolonged their residency within the lungs. More specifically, the coupling of a two-armed 40-kDa PEG chain to anti-interleukin-17A (IL-17A) F(ab’)2 and anti-IL-13 Fab’ sustained their presence within the lungs for more than 2 days. In comparison, unconjugated counterparts were mostly cleared from the lungs within a day. The prolonged pulmonary residency of the anti-IL-17A PEG40-F(ab’)2 translated into an improved efficacy in reducing markers of lung inflammation in a murine model of house dust mite-induced lung inflammation. PEGylated proteins were principally retained within the lung lumen rather than the nasal cavities or lung parenchyma. PEG increased pulmonary retention of antibody fragments through mucoadhesion and escape from alveolar macrophages rather than increased hydrodynamic size or improved enzymatic stability [130]. Conjugation of antibody fragments to PEG allowed above 50% drug loading, which is advantageous for pulmonary delivery because high drug loading limits the increase in mass load to be inhaled.

PEGylation has also been developed to prolong the retention of small inhaled drugs in the lungs [131]. Hydrolysable ester conjugates of prednisolone and 2 kDa PEG reduced prednisolone absorption rate across the pulmonary barrier by 7.7-fold following nebulization to the isolated perfused rat lung. This strategy is based on the correlation between molecular weight and pulmonary retention, as one increases, so does the other. This strategy has also been used for the preparation of cisplat-in–hyaluronan conjugates for the local treatment of lung cancer [132] and is similar to the dendrimer approach. Interestingly, the administration of inactive prodrugs of corticosteroids as PEG ester of prednisolone can reduce the incidence of local side effects in the mouth and oropharynx because inactive drugs deposited in that region will be swallowed before activation occurs [133].

4. Conclusion

The strategies to control the release of drugs locally in the lungs present both advantages and limitations. Large and porous microparticles are free-flowing and are easily delivered to the lungs using a small breath-actuated dry powder inhaler. Compared to nanocarriers, the major drawback of microparticles is their greater vulnerability to phagocytosis by alveolar macrophages. In contrast, nanocarriers escape more easily the clearance mechanisms in the lungs. In addition, their surface can be functionalized in order to affect their interactions with the lung environment and direct their fate in preferential pathways. Nanosystems offer wide possibilities in terms of structure and size. However, the increased surface area per unit volume of nanocarriers may increase the burst release of the drug and their tendency to aggregate diminishes the ease of their administration to the lungs. However, to facilitate administration to the lungs, nanocarriers can be embedded in large porous microparticles.

Compared to carrier particles, drug conjugates avoid the need for extensive formulation work and can be readily delivered by any current nebulizer device. In addition, they allow high drug loading of proteins. The drawback of drug conjugates is that they require a chemistry step.

The number of excipients approved for inhalation is currently limited. They include some sugars, some amino acids, some lipids and small size PEGs [123]. This low number of FDA-approved excipients for inhalation represents a limitation to the development of new sustained release formulations for pulmonary delivery and the use of any new excipient will require extensive toxicity studies in rodents and non-rodents. Because physiological lipids have little or no cytotoxicity, an improved toxicological profile is expected for lipid-based nanocarriers, compared to polymeric systems. Polymeric particles may cause lung
toxicity due to the slow degradation rate of some polymers such as PLGA and their possible accumulation. Among the lipid-based nanosystems, solid lipid particles may be more toxic than liposomes due to the presence of non-endogenous surfactants in their composition. As a result of their excellent safety, liposomes are one of the most extensively studied systems for controlled drug delivery to the lungs. Liposomes can be prepared with lipids endogenous to the lungs. Their composition can be adjusted to modulate drug release. They can encapsulate both hydrophilic and lipophilic compounds with high drug loading. A drawback is their limited stability under physical stress, but high dose drugs as antibiotics will require an approach offering high drug loading as liposomes or drug conjugates. Fragile molecules as proteins may not stand the stresses occurring during formulation of a carrier and may benefit from simple conjugation to PEG.

Acknowledgments
This work was supported by the Fonds de la Recherche Scientifique Médicale (Grant 3.4503.12; Fonds National de la Recherche Scientifique, Belgium). Rita Vanbever is Maître de Recherches of the Fonds National de la Recherche Scientifique.

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