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Nanotechnology and pulmonary delivery to overcome resistance in infectious diseases ☆

Fernanda Andrade [a,*], Diana Rafael [b], Mafalda Videira [b], Domingos Ferreira [a], Alejandro Sosnik [c,d,e], Bruno Sarmento [f,g,**]

[a] Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[b] iMed.UL — Research Institute for Medicines and Pharmaceutical Sciences, School of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal
[c] The Group of Biomaterials and Nanotechnology for Improved Medicines (BIONIMED), Department of Pharmaceutical Technology, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, 956 Junín St., 6th Floor, Buenos Aires CP1113, Argentina
[d] National Science Research Council (CONICET), Buenos Aires, Argentina
[e] Department of Materials Science and Engineering, Technion-Israel Institute of Tecnology, Haifa 32000, Israel
[f] CICS, Health Sciences Research Center, Instituto Superior de Ciências da Saúde Norte, Rua Central de Gandra 1317, 4585-116 Gandra, Portugal
[g] INEB-Instituto de Engenharia Biomédica, New Therapies Group, Rua do Campo Alegre 823, 4150-180 Porto, Portugal

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ABSTRACT

Used since ancient times especially for the local treatment of pulmonary diseases, lungs and airways are a versatile target route for the administration of both local and systemic drugs. Despite the existence of different platforms and devices for the pulmonary administration of drugs, only a few formulations are marketed, partly due to physiological and technological limitations.

Respiratory infections represent a significant burden to health systems worldwide mainly due to intrahospital infections that more easily affect immune-compromised patients. Moreover, tuberculosis (TB) is an endemic infectious disease in many developing nations and it has resurged in the developed world associated with the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic.

Currently, medicine faces the specter of antibiotic resistance. Besides the development of new anti-infectious drugs, the development of innovative and more efficient delivery systems for drugs that went off patent appears as a promising strategy pursued by the pharmaceutical industry to improve the therapeutic outcomes and to prolong the utilities of their intellectual property portfolio. In this context, nanotechnology-based drug delivery systems (nano-DDS) emerged as a promising approach to circumvent the limitations of conventional formulations and to treat drug resistance, opening the hypothesis for new developments in this area.

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Contents

Abbreviations: AmB, amphotericin B deoxycholate; CDs, cyclodextrins; DDS, drug delivery systems; DPI, dry powder inhalers; DSPC, distearoylphosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; GRAS, generally recognized as safe; MAC, *Mycobacterium avium–Mycobacterium intracellulare* complex; MBSA, maleylated bovine serum albumin; MDR-TB, multidrug resistant TB; MIC, minimum inhibitory concentration; mPEG–DSPE, poly(ethylene oxide)-b-distearoyl phosphatidyl-ethanolamin; MRSA, methicillin-resistant *Staphylococcus aureus*; nano-DDS, nanotechnology-based drug delivery systems; NPs, nanoparticles; OPM, *O*-palmitoyl mannan; OPP, *O*-palmitoyl pullulan; *O*-SAP, *O*-steroyl amylopectin; PAM, *p*-aminophenyl-mannopyranoside; PC, phosphatidylcholine; PC:Chol, phosphatidylcholine:cholesterol; PC:Chol:DCP, phosphatidylcholine:cholesterol:dicetylphosphate; PC:Chol:P90, phosphatidylcholine:cholesterol:P90; PLGA, poly(lactide-co-glycolide); pMDI, pressurized metered-dose inhalers; SA-CSO, stearic acid-grafted chitosan oligosaccharide; TB, tuberculosis; XDR-TB, extensively drug-resistant TB.

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* Corresponding author.
** Correspondence to: B. Sarmento,INEB-Instituto de Engenharia Biomédica, New Therapies Group, Rua do Campo Alegre 823, 4150-180 Porto, Portugal.
E-mail addresses: fersilandrade@gmail.com (F. Andrade), bruno.sarmento@ineb.up.pt (B. Sarmento).

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1. Introduction

Inhalation of compounds as a means to treat diseases is used since ancient times. The oldest reports came from China and India around 2000 BC and are related to the inhalation of smoke from burned herbal preparations based on *Ephedra sinica* or *Daturastramonium* to treat asthma, respectively [1,2]. Pedanus Discorides (40–90 AD) the Greek physician, surgeon, pharmacologist, botanist and author of *De Materia Medica* (considered the first pharmacopeia) as well as Aelius Galenus (129–199/217 AD) prescribed inhaled sulfur vapors to their patients [3]. Although the word "inhaler" was used for the first time by the English physician John Mudge in 1778 to describe his invention, the first therapeutic inhalation device is attributed to Hippocrates (460–377 BC) [1,2]. Through the years, many compounds and mixtures were proposed and used to treat different diseases using various methods for inhalation. From ceramic inhalers, to combustible powders, burning papers and liquid atomizers. Very curious and popular ways to inhale compounds in the 19th and 20th centuries were the asthma cigarettes which were withdrawn from the market in 1992 [1,3]. The inhalation of vapor from solutions of picric acid, tar, iodine or sulfuric acid was very popular in the 20th century to treat TB and other infections, especially in spas [3]. The first mentions regarding inhalation of antibiotics such as penicillin by nebulization to treat pulmonary infections were published in the 1940s [3–7]. Nowadays, inhaled drugs are preferentially administered *via* dry powder inhalers (DPI) and pressurized metered-dose inhalers (pMDI), being also used as nebulizers in hospitals.

Despite inhalation being started as a route to treat diseases constrained to the respiratory tract, with scientific and technological advances, a change in paradigm took place and over the years, inhalation has been clinically evaluated and used to treat both local and systemic diseases [8] such as asthma [9], TB [10] and other bacterial infections [11], influenza virus infection [12], fungal infections [13], cystic fibrosis [14], chronic obstructive pulmonary disease [15], diabetes [16] or cancer [17,18]. Moreover, inhalation has been also tested as a non-invasive vaccination platform [19,20].

Driven by the progress in the nanomedicine field, many researchers developed innovative formulations with improved biopharmaceutical features [21,22], allowing the pulmonary administration of many drugs that otherwise could not be administered by inhalation. Such formulations represented promising, although preliminary, alternatives to conventional inhaled formulations [23,24]. In this context, a new door has been opened in the field of inhalation therapy and new advances could be expected in the near future.

This work reviews the state-of-on overcoming drug resistance.

2. Lung and respiratory infectious diseases

Lungs and airways are a site for gas exchange and contact with the exterior, being exposed to organic, inorganic and biological components that can cause disease. There are a variety of bacterial, viral, fungal and parasitic infections that affect the lungs and can progress toward systemic infection, including pneumonia, TB, influenza, aspergillosis, among others. Infections of the lower respiratory tract are among the top three major causes of morbidity worldwide and first in low-income countries, being responsible for approximately 3.5 million deaths annually [25].

2.1. Pneumonia

Pneumonia is a common infectious pulmonary disease that has many etiological agents, including bacteria, viruses and fungi such as *Streptococcus pneumonia*, *Haemophilus influenzae* type b, *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Moraxella catarrhalis*, human respiratory syncytial virus, human parainfluenza virus, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila* or *Pneumocystis jiroveci*. It is the leading cause of death in children worldwide killing annually around 1.8 million children under the age of five years [26,27]. Nosocomial pneumonia, especially in ventilated patients, is unfortunately frequent and represents more than 50% of antibiotic prescriptions in intensive care units [28]. Also, the development of resistant strains in hospitals is causing a great deal of concern among health professionals. The therapy is chosen according to the etiological agent and the severity of the disease, the reason why it is imperative to identify the etiological agent and its sensitiveness to the available therapeutic portfolio in order to treat the patient in a rational way and to prevent the development of resistant strains.

2.2. Tuberculosis

TB is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* that primarily but not exclusively affects the lungs. It is the second leading cause of death from an infectious disease worldwide being declared a global public health emergency by the World Health Organization in 1993, a distinction never granted to any other disease. In 2011, there were around 8.7 million incident cases of TB, 1 million deaths among HIV-negative people and an additional 0.43 million deaths from HIV-associated TB [29]. The current therapy includes long-term multiple dose oral administration of various drugs, which sometimes leads to low patient compliance and therefore to the development of multidrug-resistant strains referred to as multidrug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB). The first-line drugs used in the treatment of standard TB are rifampicin, isoniazid, pyrazinamide, ethambutol and streptomycin [30,31]. At the moment new drugs are in clinical trials [32] constituting a hope for the treatment of resistant forms of the disease. In addition to the development of resistance, the difficulty to treat TB stems from the fact that when administered systemically many anti-TB drugs fail to reach the lungs or penetrate into the alveolar macrophages, the reservoir of *M. tuberculosis* [31,33]. *M. tuberculosis* persists in macrophages within a granuloma formed in the lungs of the infected hosts. Thus, traditional drug chemotherapy has serious limitations particularly the drug internalization and cytosolic availability on the infected phagocytic cells [34]. This phenomenon has driven many researchers to develop drug delivery systems (DDS) that improve the treatment of such burden.

2.3. Fungal infections

Another important question is related to the incidence of pulmonary fungal infections that has increased in the last decades partially due to the growing number of immune-compromised patients related to HIV, cancer, hematologic disorders, and organ transplantations [35]. There are a variety of fungal infections such as histoplasmosis (*Histoplasma capsulatum*), sporotrichosis (*Sporothrix schenkii*), blastomycosis (*Blastomyces dermatitidis*), coccidioidomycosis (*Coccidioides* spp.), paracoccidioidomycosis (*Paracoccidioides brasiliensis*) cryptococcosis (*Cryptococcus* spp.), aspergillosis (*Aspergillus* spp.), candidiasis (*Candida* spp.), pneumonia (*Candida* spp. and *Pneumocystis* spp.) or *Trichosporon* spp. infection that can cause pulmonary injury with different severities [35]. Actually, there are a variety of anti-fungal drugs available in the market generally administered orally or intravenously. They include amphotericin B deoxycholate (AmB), itraconazole, voriconazole, and caspofungin [35]. Among the anti-fungal drugs used to treat fungal infections, AmB is considered the gold standard therapy and it is the most commonly used therapy to treat life-threatening cases. The nephro- and cardiotoxicity of AmB is the major clinical drawback, which could lead to treatment discontinuation [36]. At the moment the marketed formulations of AmB usually used in clinical practice (*e.g.* AmBisome® (Gilead Sciences), Fungizone® (Bristol-Myers Squibb) or Abelcet® (Enzon Pharmaceuticals, Inc.)) are lipid-based DDS that present lower toxicity, while resulting in higher drug therapeutic index. AmB is extensively formulated in various nano-DDS being a proof-of-concept of the usefulness of nanomedicine in the delivery of anti-fungal drugs [37–39].

2.4. Viral infections

Regarding viral infections, another devastating problem, several viruses are responsible for acute respiratory infections namely the influenza virus, coxsackie virus, echovirus, adenovirus, respiratory syncytial virus, rhinovirus, parainfluenza virus, corona virus, and most recently human metapneumovirus, torque tenovirus, human bocavirus, polyomaviruses, avian influenza virus H5N1, and polyomavirus [40–42]. Nevertheless, the influenza virus is the main cause of viral airway infections worldwide related to severe annual epidemics, while the respiratory syncytial virus and parainfluenza virus are the most important causes of viral infection of the lower respiratory tract in children. Vaccination is the primary means of preventing and controlling viral infections, especially annual outbreaks of influenza. At the moment, few drugs are available to treat pulmonary viral infections like amantadine (Symmetrel®, Endo Pharmaceuticals, Inc.), rimantadine (Flumadine®, Forest Pharmaceuticals, Inc.) and oseltamivir (Tamiflu®, Hoffmann-La Roche) usually used in young, elderly, and other groups of patients with other serious medical conditions. Currently these drugs are administered by the oral route. Only recently, zanamivir formulated in a DPI (Relenza®, GlaxoSmithKline) was approved for the treatment of influenza by the pulmonary route. Unfortunately, there is yet no specific treatment or a vaccine available for the viruses identified recently.

3. Drug resistance

Since the 1940s antimicrobial agents have reduced the illnesses and deaths associated with infections, the discovery of penicillin being one of the greatest advances in health in the 20th century. However, through the years the accumulated overuse and misuse of anti-infectious drugs have led to the development of resistant strains. Microorganisms developed, by genetic mutations or acquisition of resistance genes, defense mechanisms that make them more dangerous and difficult to treat such as methicillin-resistant *Staphylococcus aureus* or vancomycin-resistant *Enterococcus*, resulting in prolonged illness and greater risk of death [43,44]. In addition, intracellular infections are difficult to eradicate due to low availability and activity of anti-infectious drugs inside cells. In order to treat patients with resistant strains, high doses of drugs are used that present adverse effects to healthy tissues and organs.

Despite the advances seen in the development of new antibiotics, drug resistance remains a huge public health problem. According to the World Health Organization around 440,000 new cases of MDR-TB emerge annually, causing at least 150,000 deaths worldwide [43]. Some cases of drug resistance have also been reported to HIV and influenza [44]. Nowadays, the main research focus on infectious diseases falls on the development of strategies to improve the therapeutic index of the available drugs, with nanotechnology in the forefront of the research [45].

4. Nanotechnology and pulmonary delivery

4.1. Nanotechnology in the development of drug delivery systems

The pharmacokinetics and pharmacodynamics of a drug are highly dependent on its physical and chemical features and it is influenced by the type of formulation used to deliver it. By scaling down the size of compounds, nano-DDS can modulate and improve the performance of many drugs to an extent not achievable by conventional formulations. For example, nano-DDS can be capitalized to encapsulate drugs and thereby (i) increase their solubility, (ii) protect them from degradation, (iii) enhance their epithelial absorption and increase their blood circulation time, (iv) target the drugs to specific cells/tissues/organs, releasing them in a controlled manner as a response to a specific stimulus, and (v) enhance their uptake by cells [22,23,46]. In addition, combined nano-DDS can simultaneously detect and treat a disease by encompassing both imaging and therapeutic compounds, an emerging field known as theranostics [47]. In the near future, nanomedicine could play a key role to achieve the highly desired personalized medicine.

Over the last decades, the usefulness of the design and development of nano-DDS to overcome a variety of biopharmaceutical drawbacks in the diagnosis, prevention, immunization and treatment of disease has been intensively explored by a large number of research groups worldwide, leading to a great number of scientific articles published in international journals. Moreover, it has generated a profuse intellectual property platform. However, and despite the fact that nanomedicine began as a discipline almost half a century ago, only a few nano-DDS have found their way to the market [48]. This phenomenon could be explained by the lack of financial profitability, consumer distrust and the lack of confidence due to poor information/education, ineffective regulation of new and generic products, and weak patent protection [49]. At the moment, regulatory agencies are in process of developing specific guidelines and regulations regarding nanotechnology-based products in order to develop new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of such products [50,51]. Nonetheless, the relatively few marketed nano-DDS have been successful in their respective therapeutic areas. According to BCC Research, the global nanomedicine market has been growing steadily, reaching a value of $72.8 billion in 2011, with anticancer agents as the market leader and it is expected to increase at an annual growth rate of 12.5% until 2016 reaching $130.9 billion [52].

In this context, many research groups have consolidated the concept of pulmonary delivery of drugs using nano-DDS. Preliminary results are promising and it is expected that the clinical relevance of such products could be proven in clinical trials [18,23,53]. The therapeutic efficiency of anti-infectious drugs can be improved by enhancing their solubility, pulmonary accumulation and further intracellular delivery thus increasing the local concentration of drugs, reducing the dose and frequency of administration and minimizing at the same time the drugs' systemic side effects [33,54]. Consequently, patient compliance is augmented avoiding the development of multidrug-resistant strains. Still, several issues such as large scale production, batch-to-batch reproducibility, variable lung deposition pattern, or cost-effectiveness

balance of the treatment need to be addressed and optimized in order to ensure the bench-to-bedside translation of inhalatory nano-DDS.

4.2. Inhalation as a platform to delivery of drugs

As stated above, inhalation serves as a platform for the delivery of drugs aimed to display both local and systemic activity. The same anatomical and physiological characteristics that can make airways susceptible to infection by external pathogens can be exploited for drug delivery. The respiratory system provides a non-invasive route of delivery with a large surface area, a thin epithelial barrier, extensive blood supply and blood flow, and lower enzymatic activity compared to other organs in the body (*e.g.*, liver and gastrointestinal tract). Also, first-pass metabolism is avoided by pulmonary administration, which is especially useful for drugs that suffer high hepatic metabolism [53,55]. After inhalation, particles will be transported with the inhaled air through the respiratory tract until they will be deposited or exhaled. Among the features that influence the behavior of inhaled particles in the airways, the mean aerodynamic diameter (Eq. (1)) plays an important role as it governs the particle deposition pattern.

$$dae = deq\sqrt{\frac{\rho p}{\rho o X}}. \quad (1)$$

Where d*eq* is the diameter of an equivalent volume sphere of unit density, ρp and ρo are particle and unit densities and X is the dynamic shape factor.

It is commonly accepted that the optimal mean aerodynamic diameter range for particle deposition in the lower airways is 1–3 μm. Particles larger than 5 μm undergo deposition in the oropharynx and they are consequently swallowed, while particles smaller than 1 μm are likely exhaled [55,56]. However, studies in the area of environmental toxicology showed that nanoparticles with sizes ranging between 1 and 100 nm could also randomly deposit to a great extent in the lower airways (Fig. 1) [57]. The mechanisms involved in particle deposition also depend on the anatomy of the airway region and they include inertial impaction, sedimentation and diffusion (Fig. 2) [58,59].

4.3. Excipients used in the development of inhalatory formulations

Besides drugs, pharmaceutical excipients constitute an integral part of pharmaceutical formulations. They provide physical, chemical or microbiological stability, bulk properties that improve handling and metering, while controlling the mechanical and pharmaceutical properties of formulations such as release and permeation [56,60]. At the moment, only a small number of excipients are authorized for pulmonary delivery, but a variety of new excipients is under evaluation. Since lungs have limited buffer capacity, only compounds that are biocompatible or endogenous to the lung and that are easily metabolized or cleared can be used in inhaled formulations [60]. Since formulations for nebulization are liquid solutions or suspensions, the common excipients used are salts (*e.g.*, NaCl) to adjust the osmolarity (300 mosmol/L), HCl, NaOH, phosphates to adjust the pH to neutrality, and surfactants such as polysorbates, sorbitan monostearate, oleic acid and soy lecithin to facilitate the formation of liquid droplets. Ethanol can be used as co-solvent and permeation enhancer only in small concentration due to its irritation potential. Preservatives, antioxidants or chelating agents such as parabens and benzalkonium chloride, ascorbic acid, and ethylenediaminetetraacetic acid (EDTA) can also be used to enhance stability [56]. The excipients used in pMDI are similar to those found in preparations for nebulization except for the gas propellants. The most widely excipient used as propellant in pMDI is hydrofluoroalkane, a non-toxic, non-flammable, and chemically stable gas without carcinogenic or mutagenic effects. Due to the absence of ozone-depleting properties, hydrofluoroalkane has been replacing chlorofluorocarbon-based propellants [61]. DPI were developed as a response to the limitations regarding stability and environmental aspects of pMDI and nebulizers [60]. Being solid dispersions of drugs blended with a coarse carrier particle, the efficacy of a DPI depends on powder technology and particle engineering. In DPI, the coarse carrier particle is the major component of the formulation (>95%, w/w). It provides bulk properties and reduces the cohesion forces between drug particles, facilitating the aerosol dispersion and defining deposition pattern [62]. Lactose is the main excipient used as coarse and larger carrier particles and, to a lower extent, as cryoprotectant when particles are prepared using lyophilization. A variety of inhalation grade lactoses with a narrow particle size distribution like Lactohale 100®, Inhalac 230® or Respitose ML001® are commercially available. Other sugars such as glucose, trehalose and mannitol are also used as cryoprotectants and coarse carriers [56]. Magnesium stearate is approved for inhalation to protect the drug from moisture and to reduce cohesion and adhesion between particles [63].

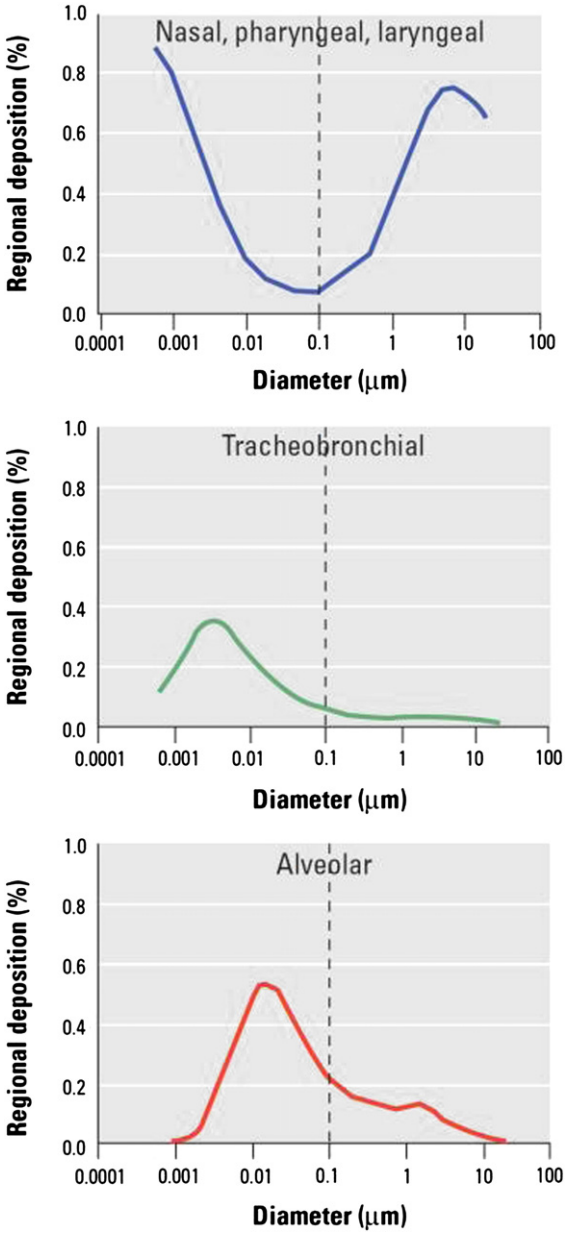

Fig. 1. Regional deposition pattern of particles according to their aerodynamic diameter. Adapted from reference [57] with permission of the National Institute of Environmental Health Sciences.

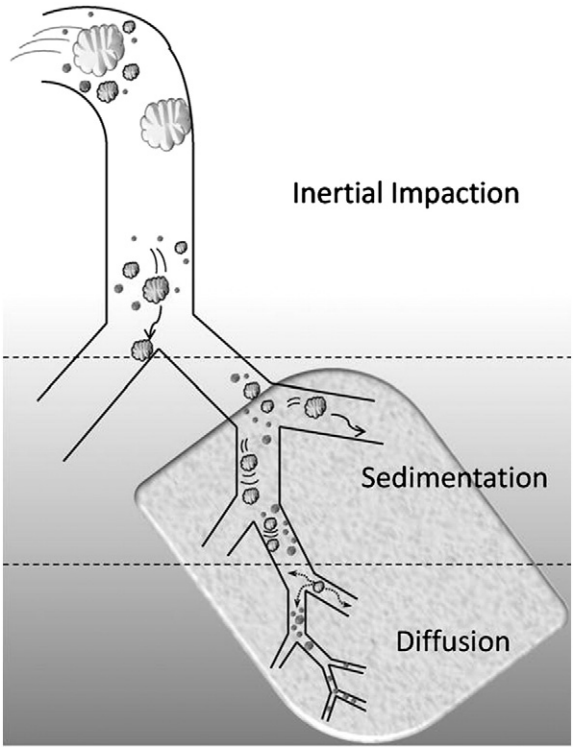

Fig. 2. Mechanisms involved in particle deposition in the different regions of airways. Reproduced from reference [58] with permission of Elsevier.

Regarding inhaled nano-DDS, most of the components are generally not approved for inhalation. In this case, new excipients proposed for inhalatory formulations need to pass through the entire process of safety evaluation, including complete *in vitro* toxicological evaluation and *in vivo* assessment of non-clinical and clinical safety prior to licensing. Unfortunately, there is a lack of specific regulatory guidance regarding the toxicological assessment of excipients for inhalation [64–66]. Excipients generally recognized as safe (GRAS) or those approved for other routes of administration need a more limited number of experiments for safety evaluation, their acceptance by regulatory agencies being usually easier. This is the case of phosphatidylcholine (PC), a lipid surfactant and one of the constituents of lung surfactant that is commonly used in the production of liposomes. Studies show that PC-based liposomes do not affect or slightly decrease the viability of human A549 alveolar cells after 24 h of exposure. The effects on cell viability are dependent on the PC derivate and the concentration used [67]. Other compounds used in the development of nano-DDS such as dextran, alginate, carrageenan or gelatin also possess GRAS status. It should be mentioned that that regulations apply not only to the material itself but also to its source. Thus, chitosan obtained from *Aspergillus niger* is in the process to obtain GRAS status. Chitosan does not present significant toxicity to pulmonary tissue and cells after inhalation [68,69]. In fact, some studies showed some protective effect against oxidative stress [68]. Poly(lactide-co-glycolide) (PLGA) is another biodegradable polymer extensively used in the production of nano-DDS. The cytotoxicity of PLGA nanoparticles with different coatings and surface charges was assessed. Results showed that the cytotoxicity to human bronchial Calu-3 cells was very limited, with the absence of inflammatory response [70]. Cyclodextrins (CDs) have been tested as complexing agents and excipients of inhalatory formulations. Various approved formulations containing CDs such as Voltaren® (Novartis), Clorocil® (Laboratório Edol), Brexin® (Chiesi Farmaceutici) or Vfend® (Pfizer), are used daily in the clinical practice for administration routes other than inhalation [71].

Recently, carrier-free formulations that presented good aerosolization properties and deposition patterns have been developed and proposed as promising inhalatory formulations [72,73]. This "carrier-less" strategy avoided the need for long and expensive safety studies, facilitating the authorization by regulatory agencies. At the moment, there are commercially available carrier-free DPI composed by agglomerates of pure terbutalin and budesonide particles, namely Bricanyl Turbohaler® (AstraZeneca) and Pulmicort Turbohaler® (AstraZeneca), respectively.

4.4. Inhalation devices

Apart from the characteristics of particles and formulations, inhalatory administration demands an inhaler device that produces an appropriate aerosol. There are numerous devices available with distinct properties and specifications that are more appropriate than others for each type of formulation (Fig. 3). Since the device greatly influences the particle aerosolization and deposition pattern, the choice of the appropriate device for a specific formulation is one of the most time-consuming and challenging stages of the development phase and it is imperative to ensure the drug efficacy [62,74]. For example, Alexander et al. tested the aerosolization efficacy of Ambisome® (Gilead Sciences) using different nebulizers and they found differences among all of them [75]. The inhaler device needs to be accurate, small, easy to handle, discreet, and user friendly in order to be accepted by both clinicians and patients. One of the claimed reasons for the market failure of Exubera® (Pfizer) was low patient compliance partly due to the complex inhaler device [76]. Owing to the great therapeutic potential of this alternative administration route, the last decades witnessed the development of devices with greater pulmonary deposition fractions of approximately 40–50% of the nominal dose as compared with the low levels between 10 and 15% verified in the past [61].

However, it is worth mentioning that this is a growing and dynamic field and innovative technologies are emerging. For example, power-assisted devices activated by vibration [77,78] or pneumatic technology [79], called active inhalers, have been developed for the delivery of systemically active drugs that have narrow therapeutic windows and for inhalatory nano-DDS [60]. Also, the development of adjusted inhalers that present specific inhalation patterns that are useful for the treatment of certain pulmonary diseases can be given attention [61]. It is also important to consider the cost of the device and its impact on the final product price. A balanced price/therapeutic efficacy relationship is a crucial factor that can limit the clinical application of a certain product.

5. Nano-based strategies for delivery of anti-infectious drugs and to overcome drug resistance

Severe infections are frequently induced by resistant strains and/or intracellular pathogens, thus making their treatment complex. Bacterial pathogens such as *M. tuberculosis*, *Mycobacterium avium*, *Mycobacterium intracellulare*, and *Legionella pneumophila*, responsible for different pulmonary diseases, present the capacity to overcome cellular defense mechanisms, infecting the cells (often phagocytic ones) and turning them into reservoirs [80]. Conventional anti-infectious drugs display some drawbacks limiting the effective and timely eradication of intracellular pathogens (Fig. 4) [80]. Also, some drugs display limited bioavailability in the target microorganism to exert its therapeutic effect. In this framework, nano-DDS could overcome such limitations and improve the therapeutic index and clinical relevance of such drugs. Liposomal amikacin [81,82], kanamycin [83], streptomycin [84,85], gentamicin [86], vancomycin and teicoplanin [87] present enhanced *in vitro* and/or *in vivo* efficacy by reducing the viable bacteria counts of *Mycobacterium avium–Mycobacterium intracellulare* complex (MAC), *M. tuberculosis*, *Klebsiella pneumonia* and methicillin-resistant *S. aureus* (MRSA). Nevertheless, in some cases, the pulmonary

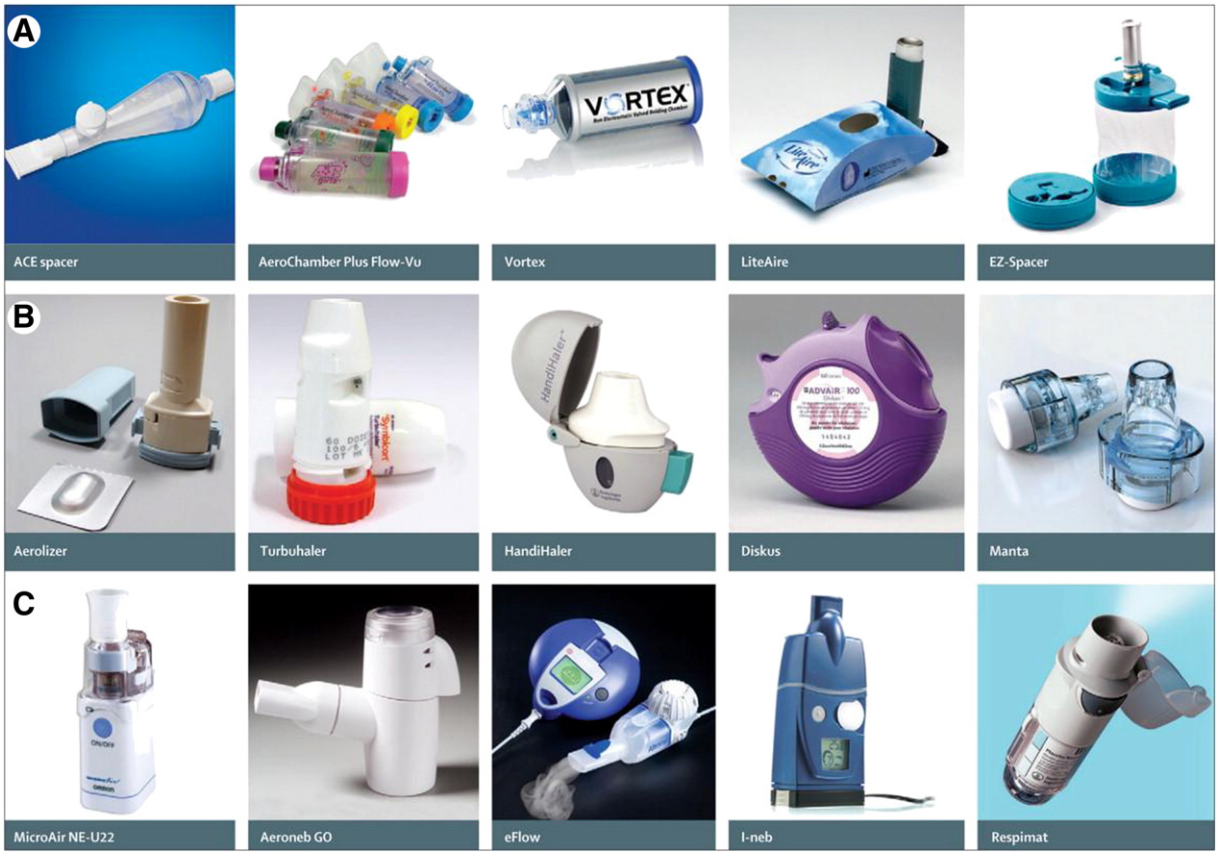

Fig. 3. Examples of inhaler devices available. Reproduced from reference [61] with permission of Elsevier.

biodistribution of the different drugs was low or absent due to the poor lung targeting and accumulation properties of some of these formulations [84,85]. This could be overcome by the development of particles targeted to the lung (*e.g.* MiKasome®, NeXstar Pharmaceuticals, Inc.) [88] or by the localized delivery by inhalation.

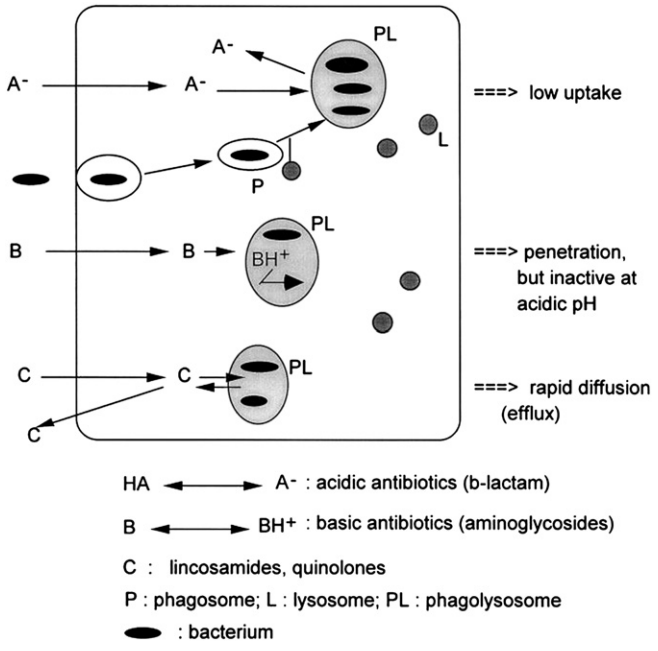

Fig. 4. Schematic of traffic of free antibiotics into phagocytic cells. Reproduced from reference [80] with permission of Elsevier.

Due to their membrane-like structure, liposomes can fuse with the microorganism's membranes and deliver high drug cargos into the cytoplasm, saturating their drug efflux pumps [45]. On the other hand, micelles of poloxamers present the capacity to inhibit the efflux systems [89]. Other nanoparticles can be adsorbed to the microorganism's surface and serve as a depot for the encapsulated drug or to be taken up by infected cells, increasing the availability of the anti-infectious drug and overcoming drug resistance [45]. It is also possible to target the nanoparticles to surface receptors of microorganisms to further improve their therapeutic efficacy (*e.g.* lectin-decorated nanoparticles).

Many inhaled anti-infectious drugs formulated in microparticle systems have proved the usefulness of inhalation as an effective route of administration to treat infectious diseases. Inhaled rifampicin-loaded PLGA microspheres efficiently reduced the pulmonary burden of *M. tuberculosis* and presented lower toxicity compared to the free drug [90,91]. Also, ofloxacin-loaded hyaluronan microspheres administered intratracheally were more efficiently delivered into the lungs and underwent greater uptake by alveolar macrophages than intratracheal ofloxacin solution and i.v. or oral ofloxacin/hyaluronan microspheres [92].

Proof-of-concept studies of inhaled nano-DDS to treat lung and systemic infections were reported by several research groups (Table 1). However, as mentioned above, the properties of the encapsulated drug and the pharmaceutical excipients on the one hand, and the conditions of the production and the aerosolization process on the other hand greatly affect the performance of the final formulation. Thus, the comparison and the extrapolation of data between different studies are difficult. For example, distearoylphosphatidylcholine (DSPC)/cholesterol (4:1) liposomes intended to inhalation and prepared using the same conditions efficiently encapsulated isoniazid, pyrazinamide and ethionamide, but did not encapsulate rifampicin and streptomycin [93]. Conversely, in another study, rifampicin was efficiently incorporated into DSPC/cholesterol (2:1) liposomes using a similar preparation protocol [67].

Table 1
Examples of nano-DDS developed for administration of anti-infectious drugs by inhalation.

Drug	nano-DDS	Organism	Device	Reference
Amphotericin B	Chitosan-based micelles	*Candida albicans*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Cryptococcus neoformans*	Nebulizer	[38]
	Functionalized liposomes	Not tested	pMDI	[37]
Ciprofloxacin	Functionalized liposomes	Various (pharmacokinetic/pharmacodynamic)	pMDI	[119]
	Liposomes	Not tested	DPI	[127]
	Liposomes	Not tested	DPI	[102]
	Liposomes	*Francisella tularensis*	Nebulizer	[103]
CM3	Liposomes	Not tested	DPI	[102]
Isoniazid, rifampicin and pyrazinamide	Alginate/chitosan nanoparticles	*Mycobacterium tuberculosis*	Nebulizer	[97]
	Solid lipid nanoparticles	*Mycobacterium tuberculosis*	Nebulizer	[98]
	Poly (lactide-co-glycolide) nanoparticles	*Mycobacterium tuberculosis*	Nebulizer	[99]
	Lectin-poly (lactide-co-glycolide) nanoparticles	*Mycobacterium tuberculosis*	Nebulizer	[100]
Itraconazole	Polysorbate 80:poloxamer 407 nanoparticles	*Aspergillus fumigatus*	Nebulizer	[109–112]
	Mannitol:lecithin nanoparticles	Not tested	Nebulizer	[114]
Polymyxin B	Liposomes	*Pseudomonas aeruginosa*	Intratracheally	[106]
Rifampicin	Cyclodextrin complexes	*Acinetobacter baumannii*	Nebulizer	[128]
	Chitosan-coated liposomes	Not tested	Nebulizer	[96]
	Liposomes	*Mycobacterium avium*	Nebulizer	[122]
	Functionalized liposomes	*Mycobacterium smegmatis*	pMDI	[117]
	Poly-(ethylene oxide)-b-distearoylphosphatidyl-ethanolamine micelles	Not tested	Nebulizer	[94]
	Poly(lactic-co-glycolide) nanoparticles	Not tested	DPI	[123]
	Liposomes	Not tested	DPI	[95]
Silver–carbene complex	L-tyrosine polyphosphate nanoparticles	*Pseudomonas aeruginosa*	Nebulizer	[108]
Tobramycin	Liposomes	*Pseudomonas aeruginosa*	Intratracheally	[104]
	Liposomes	*Burkholderia cepacia*	Intratracheally	[105]
Voriconazole	Cyclodextrin complexes	*Aspergillus fumigatus*	Nebulizer	[115]

5.1. Bacterial infections and TB

Due to its low water solubility, rifampicin is the focus of studies regarding encapsulation into different nanocarriers. Poly(ethylene oxide)-b-distearoylphosphatidyl-ethanolamine (mPEG–DSPE) micelles were proposed as carriers for rifampicin, although their therapeutic efficacy was not assessed [94]. Rifampicin was entrapped with high encapsulation efficiency into these micelles that sustained its release over 3 days *in vitro*. Formulations presented a fraction of fine particles of approximately 40% after nebulization [94], an acceptable value that can be increased through system optimization. In another study, liposomal DPIs were developed and assessed as a platform for rifampicin inhalation [95]. The cryoprotectant used during the freeze-drying process influenced the aerosolization properties of the formulations. Between trehalose, mannitol and lactose, mannitol presented the best results regarding fine particle fraction and mean aerodynamic diameter [95]. The coating of the liposomes with muco-adhesive polymers such as chitosan improved the encapsulation efficiency of rifampicin but it distinctly affected the nebulization properties of the formulations as well as their cytotoxicity, that were intimately associated with the composition [96]. Nevertheless, the fine tuning of the liposome composition and considering that the muco-adhesiveness of the systems was enhanced [96], it was expected that the lung accumulation of rifampicin, and thus its therapeutic effect, would be increased, making this a promising strategy to improve the therapeutic index of inhaled rifampicin. Aiming to avoid the administration of multiple medicines on a daily basis, Zahoor et al. developed alginate-chitosan NPs loaded simultaneously with isoniazid, rifampicin and pyrazinamide [97]. Around 80% of the nebulized formulation presented a mean aerodynamic diameter of 1.1 ± 0.4 μm, suitable for inhalation. Biodistribution studies performed in guinea pigs showed the presence of the drugs in plasma for a period exceeding 10 days, the drugs being detected in the lungs, liver and spleen in concentrations that were above the minimum inhibitory concentration (MIC) for up to 15 days (Fig. 5). In contrast, free drugs administered by the same route were cleared after 24 h. In addition, inhalation of nanoparticles in a regimen of one administration every 15 days for 45 days (3 administrations) presented the same localized and systemic therapeutic activity against *M. tuberculosis* in an animal model of the infection as the combination of the free drugs administered *p.o.* daily for 45 days (Table 2). Furthermore, there was no evidence of any biochemical hepatotoxicity [97]. Similar results were obtained by the same group using SLNs [98], PLGA [99] and wheat germ agglutinin-PLGA [100] nanoparticles containing rifampicin, isoniazid, and pyrazinamide, though these formulations required higher plasma residence time and different dose regimens. The existence of receptors for lectins in the alveolar epithelium could potentiate the absorption of drugs encapsulated into lectin-functionalized nanoparticles [100]. These modified systems emerge as a promising approach because they would allow the reduction of the dosing frequency and, by doing so, the improvement of patient compliance. At the same time, the cost–benefit balance should be thoroughly assessed for each one of them to ensure patient affordability. Other nano-DDS loaded with kanamycin [101], isoniazid, pyrazinamide, streptomycin, rifampicin or ethionamide [67,93] have been developed. However, their aerosolization properties and therapeutic efficacy in TB were not assessed.

As stated above, nosocomial respiratory infections challenge the currently available therapy and represent a serious problem in healthcare units. The encapsulation of antibacterial drugs and their pulmonary administration improve their therapeutic index and leads to the reduction of the dose required to reach the local MIC. A liposomal DPI of ciprofloxacin presenting a mean aerodynamic diameter of 2.8 ± 1 μm and 60% of fine particle fraction appeared as a possible formulation for the treatment of respiratory infections by the inhalatory route. Similar results were obtained by Desai et al. using liposomes loaded with ciprofloxacin and CM3, a peptide with antimicrobial properties [102]. In another study, inhaled ciprofloxacin/liposomes provided protection against a lethal pulmonary tularemia murine model. Unlike untreated (control) and free inhaled ciprofloxacin treated mice, one single dose of the liposomal formulation resulted in at least 86% of survival after 14 days [103]. Inhaled liposomal tobramycin was therapeutically more effective than the conventional inhalatory formulation (Tobi®, PathoGenesis Canada Ltee) against *Pseudomonas aeruginosa* in a multiple

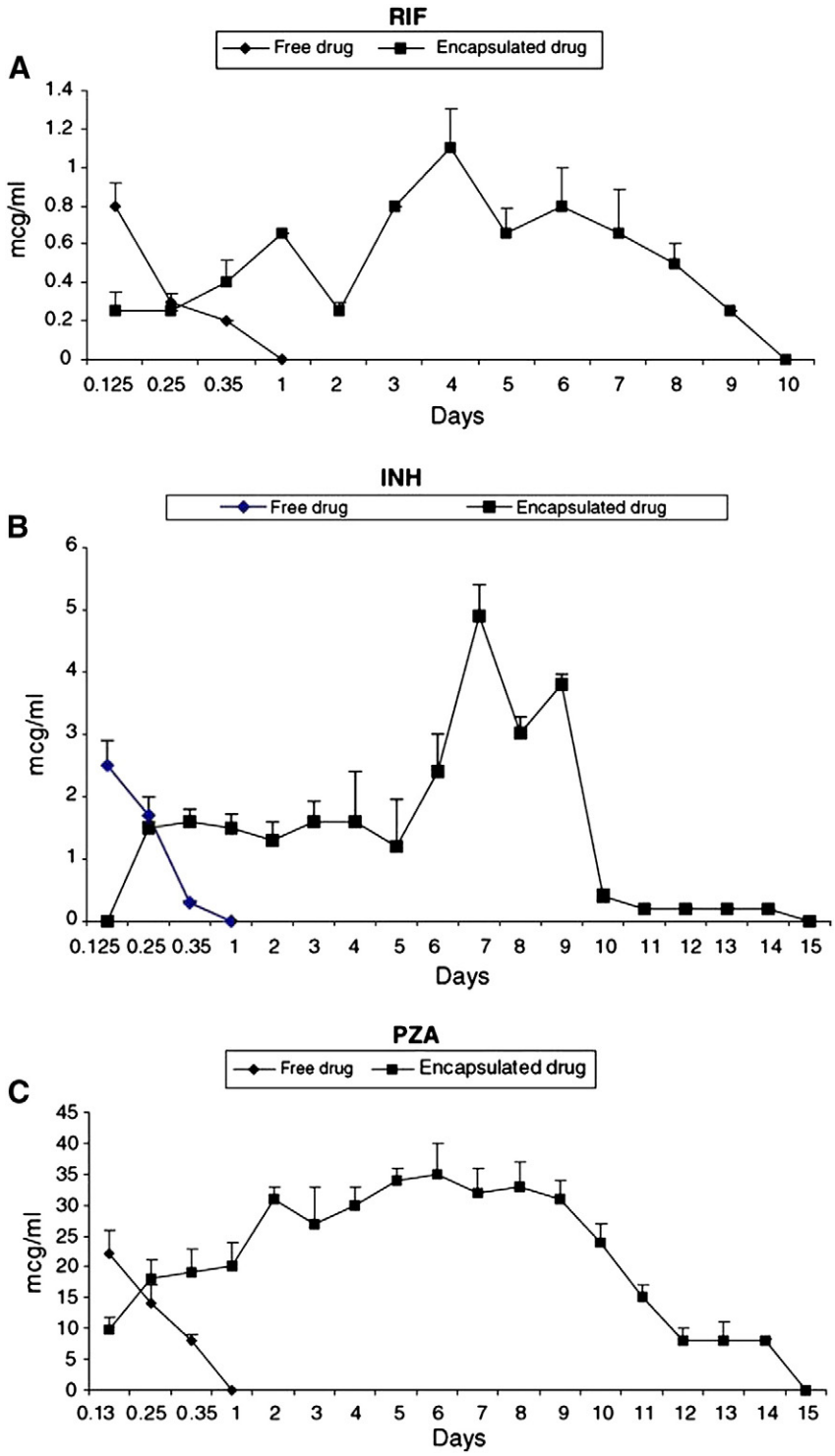

Fig. 5. Plasma drug profile following a single nebulization of antitubercular drug loaded with alginate/chitosan nanoparticles and free drugs to guinea pigs (mean ± S.D.) (n = 6). RIF, rifampicin; INH, isoniazid; PZA, pyrazinamide. Reproduced from reference [97] with permission of Elsevier.

dose treatment conducted in infected rats [104]. These findings were explained by differences in the pulmonary pharmacokinetic profile of the different formulations. Liposomes display a greater area under curve (3890 ± 560 μg h/lungs) and elimination half-life time (34.4 ±5.0 h) with respect to the conventional formulation (663 ± 89 μg h/lungs and 14.0 ± 4.0 h) [104]. Similar results were obtained in the treatment of rats infected with *Burkholderia cepacia* using the same formulation [105]. In another study, inhaled liposomal polymyxin B presented higher pulmonary levels of the drug (42.6 ± 6.2 μg/paired lungs) compared to the free drug (8.2 ± 0.4 μg/paired lungs) and reduced the lung injuries resulting from *P. aeruginosa* infection in rats [106]. The same formulation was shown to possess lower MIC values for various Gram-negative bacteria including resistant strains of *P. aeruginosa* [107]. Also, a silver–carbene complex with antimicrobial properties was encapsulated in L-tyrosine polyphosphate nanoparticles and nebulized to mice infected with *P. aeruginosa*. The treatment reduced the number of bacteria in lungs and spleen and increased the survival of the animals [108].

Table 2
Chemotherapeutic efficacy of aerosolized alginate nanoparticles encapsulating antitubercular drugs against experimental tuberculosis in guinea pigs. Reproduced from reference [97] with permission of Elsevier.

Group	Log_{10} colony-forming units[a]	
	Lung (right caudal lobe)	Spleen (whole organ)
Untreated controls	5.8 ± 0.1	5.9 ± 0.1
Empty alginate nanoparticles every 15 days, aerosol (three doses)	5.8 ± 0.3*	5.9 ± 0.1
Drug-loaded alginate nanoparticles every 15 days, aerosol (three doses)	<1.0[b]	<1.0[b]
Free drugs daily, orally (45 doses)	<1.0[b]	<1.0[b]

[a] Results are based on visible growth of *Mycobacterium tuberculosis* on Middlebrook 7H10 agar on day 21 post inoculation (mean ± S.D.) (n = 5–6).
[b] Value <1.0 indicates no detectable CFUs following the inoculation of 50 μL of neat and 1:10 diluted tissue homogenates.
* $P > 0.05$ according to ANOVA.

5.2. Fungal infections

Recently, stearic acid-grafted chitosan oligosaccharide (SA-CSO) micelles were developed for the pulmonary administration of AmB [38]. AmB-loaded micelles presented the same antifungal activity of Fungioze® but lower toxicity. The formulations were efficiently nebulized using an air-jet nebulizer presenting up to 52% of fine particle fraction [38]. Also, in the treatment of fungal infections, itraconazole: polysorbate 80:poloxamer 407 nanostructured aggregates administered by inhalation to mice presented higher lung concentrations, lung-to-serum ratios, improved survival of infected animals and lower toxicity than orally administered itraconazole formulations, in a 12 day study [109–112]. No signs of lung inflammation or changes in pulmonary histology were detected [113]. It is worth stressing that a lower drug dose was required to achieve lung and serum therapeutic levels using inhaled formulation compared to oral administration [110]. Itraconazole nanostructured aggregates composed of mannitol and lecithin prepared by an ultra-rapid freezing technique also presented promising features such as aerosolization suitable for deep lung delivery, high lung deposition and systemic absorption, making them a promising approach to treat invasive fungal infections [114]. Other studies assessing the encapsulation of newer antifungal agents, *e.g.*, voriconazole-sulfoalkyl ether/CD complexes to treat pulmonary fungal infections by inhalation were patented [115]. Nebulization of complexes produces a fine particle fraction of about 70% of the nebulized droplets [115]. Apart from the enhanced aqueous solubility of voriconazole, the formulation led to a pronounced increase of the accumulation in lungs and to a more limited clearance from plasma, these phenomena increase its therapeutic efficacy in *Aspergillus fumigatus*-infected animals; greater survival extents and reduced lung damage were observed. CDs are proposed not only as the carrier in nano-DDS but also as therapeutic agent in the treatment pulmonary infections. Due to its structure, the Per-6-[(N^{α}-Boc-L-ornithinyl)amino]-β-cyclodextrin displayed the capacity to bind to α-hemolysin. In addition to the *in vitro* results that showed the prevention of alpha-hemolysin-induced lysis of human alveolar epithelial cells, this CD prevented the mortality in a *S. aureus* pneumonia murine model [116]. However, the compound was administered intravenously, being necessary to perform pulmonary administration to determine its feasibility as an inhalatory therapeutic agent.

6. Macrophage targeting in the treatment of infections

Being a first-line defense mechanism against microorganisms entering the lung *via* the airways and, consequently, reservoirs of many pathogens, alveolar macrophages have drawn special attention as a relevant clinical target. Various nano-DDS of different drugs have been developed to selectively deliver the therapeutic cargo to alveolar macrophages, presenting positive results in the treatment of both fungal and bacterial infections [34,37,117]. The targeting of drugs to macrophages could be achieved by tailoring physicochemical properties of the carriers like size, hydrophilicity, composition, surface charge and surface ligands [118]. Different ligands such as galactomannan, maleylated bovine serum albumin (MBSA), *O*-steroyl amylopectin (*O*-SAP), *O*-palmitoylmannan (OPM), *p*-aminophenyl-mannopyranoside (PAM), and *O*-palmitoylpullulan (OPP) were used to promote the targeting and uptake of particles by macrophages *via* receptor-mediated endocytosis using for example mannose receptors and other lectin-like receptors [37,117,119]. In this context, Vyas and Khatri published an interesting review regarding the use of liposomes as delivery systems to alveolar macrophages [120]. Poly(ε-caprolactone)-*b*-poly(ethyleneglycol)-*b*-poly(ε-caprolactone) micelles and chitosan nanoparticles functionalized with hydrolyzed galactomannan were taken up by murine macrophages and promoted increased intracellular levels of rifampicin to a higher extent than non-functionalized particles [121]. Rifampicin-loaded liposomes produced as pressurized formulations were efficiently aerosolized and deposited in the lower airways. The encapsulation of rifampicin led to a reduction of the *Mycobacterium smegmatis* viability inside macrophages from 45.7 ± 4.7% to 21.6 ± 3.1% for the free drug and phosphatidylcholine: cholesterol:dicetylphosphate (PC:Chol:DCP) liposomes, respectively (Fig. 6). This effect was further increased by anchoring MBSA (10.9 ± 2.1%) and *O*-SAP (7.1 ± 1.6%) to the surface of the liposomes [117]. Authors speculated that the ideal 0% of viability could be reached by a repeated dosing regimen. Being taken up by macrophages, ligand-modified liposomes accumulated in the lungs for more prolonged time and they were cleared more slowly through the blood stream (as suggested by *in vivo* biodistribution studies in Wistar rats), exerting their effect locally and more efficiently [117]. This approach appears to be a feasible strategy for the treatment of pulmonary TB.

Passive targeting to alveolar macrophages is also possible, although it is expected that functionalized liposomes will present better results as they will be taken up selectively by the cellular reservoir of the bacillus. Thus, rifampicin-loaded phosphatidylcholine:cholesterol:P90 (PC:Chol:P90) liposomes were stable in the presence of mucus and underwent deposition in the lower airways, when administered to rats. Most importantly, in vitro studies showed that liposomes enhanced the uptake of rifampicin by alveolar macrophages and inhibited the growth of intracellular *Mycobacterium avium* even when a lower dose of rifampicin was used [122]. Rifampicin-loaded PLGA nanoparticles also result in higher uptake *in vitro* and *in vivo* by alveolar macrophages when compared to microspheres of similar composition [123], indicating that the size of the particles influences the cellular uptake.

The pulmonary administration and antibacterial activity of ciprofloxacin-loaded liposomes against the intracellular bacteria were also assessed [119]. The uptake of ciprofloxacin by alveolar macrophages and the pulmonary accumulation after inhalation were higher for liposomal formulations than for the drug solution, the effect being greater for PAM-modified liposomes [119]. The antibacterial activity against various intracellular bacteria such as *Chlamydophila pneumonia*, *Legionella pneumophila*, *Listeria monocytogenes* and *Francisella tularensis* as estimated by pharmacokinetic/pharmacodynamic analyses, and it was higher using liposomal systems. This enabled a significant reduction of the administered dose [119]. Yet, efficacy studies using the pathogens need to be performed to support the results.

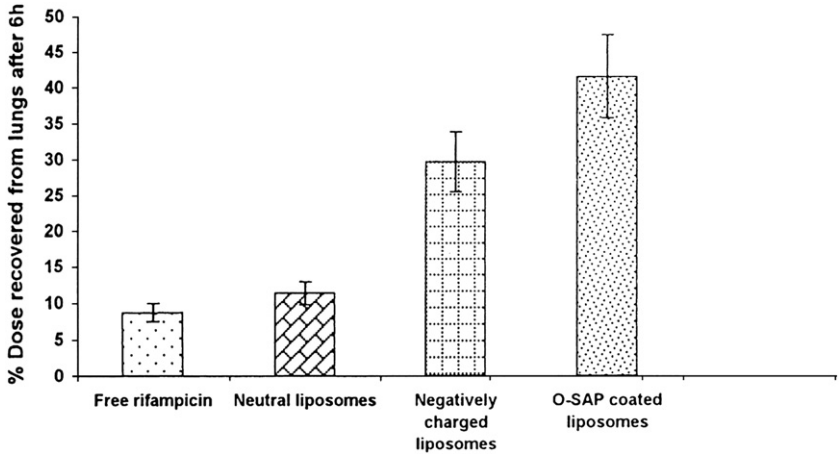

Fig. 6. Relative lung retention of rifampicin formulations after 6 h pulmonary administration to rats (mean ± S.D.) (n = 3). Reproduced from reference [117] with permission of Elsevier.

Regarding fungal infections, OPM and OPP were used as ligands recognizable by mannose receptors to target AmB-loaded phosphatidylcholine:cholesterol (PC:Chol) liposomes to alveolar macrophages [37]. The PC:Cholmolar ratio of 7:3 presented the best relationship between AmB encapsulation efficiency and hemolysis-related toxicity. However, increasing the percentage of cholesterol in the liposome led to a decrease in the encapsulation efficiency and toxicity. pMDIs prepared with these formulations showed acceptable percentage of particles deposited in lower airways *in vitro*, although smaller than the free drug solution. Additionally, after administration to rats, liposomes accumulated more effectively in the lungs and prolonged the release with respect to the free drug. The presence of OPM and OPP improved the pulmonary accumulation of AmB, showing better results than the OPM-anchored liposomes (Fig. 7) [37]. Efficacy studies regarding these formulations are needed for further evaluation.

Another relevant discovery is related to tuftsin, a tetrapeptide of Thr–Lys–Pro–Arg, that specifically binds to macrophages potentiating its phagocytosis, immunogenic response and bactericidal activity [124]. Besides its antimicrobial activity, grafting tuftsin to the surface of liposomes provides a useful delivery system for many drugs to treat among other infections TB [125], leishmaniasis [39] and aspergillosis [126]. Still the pulmonary administration of such formulations needs to be assessed.

7. Conclusion

Inhalation of drugs to treat both local and systemic pathologies has gained much attention in the last decades. The anatomo-physiological characteristics of the respiratory system make them a promising non-invasive administration route that minimizes systemic exposure and adverse effects. However, despite the advantages presented over other administration routes, inhalation requires formulations with fine tuned properties (*e.g.*, aerodynamic properties) as well as the implementation of special delivery devices that make its development and production under an industrial setup many times more difficult and expensive. This explains the small number of inhalatory formulations available in the market. Nonetheless, scientific developments in the fields of both drug delivery and inhalation device technology over the last years have increased interest in the pulmonary delivery of drugs. One of the main developments has been in the field of nanotechnology, where a multitude of nano-DDS have been developed and tested.

The main advantages of the abovementioned DDS include the sustained release of drugs over longer periods of time, prevention of premature drug degradation, improvement in drug absorption, enhancement of tissue penetration and accumulation and reduction of drug toxicity. Regarding anti-infectious drugs, the achievement of the same therapeutic index using fewer drug doses as well as a lower dosing frequency is definitely advantageous since this improves patient compliance and adherence to the treatment. In addition, targeted systems to alveolar macrophages improve bactericidal activity on intracellular pathogens, overcoming the limitations of conventional formulations. Therefore, inhalatory nano-DDS represent a promising approach and a hope in the treatment of respiratory infections and can be especially valuable to fight intrahospital multidrug resistant strains.

Although the results obtained so far are too preliminary, the steady increase in the development of such systems and the proof of their therapeutic relevance in preclinical models and clinical trials could be certainly envisaged in the near future.

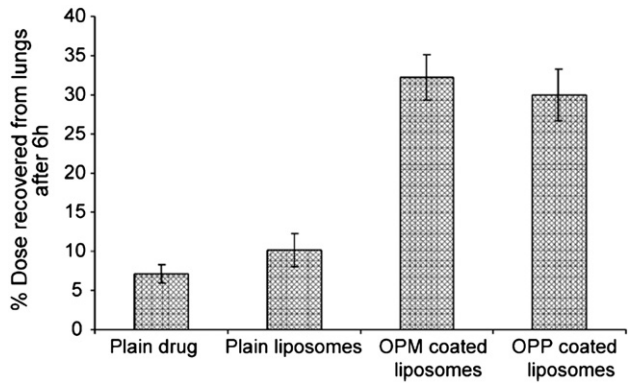

Fig. 7. Relative lung retention of AmB formulations after 6 h pulmonary administration to rats (mean ± S.D.) (n = 3). Reproduced from reference [37] with permission of Elsevier.

Conflict of interest and acknowledgment

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References

[1] P.J. Anderson, History of aerosol therapy: liquid nebulization to MDIs to DPIs, Respir. Care 50 (2005) 1139–1150.
[2] M. Sanders, Inhalation therapy: an historical review, Prim. Care Respir. J. 16 (2007) 71–81.

[3] J.F. Dessanges, A history of nebulization, J. Aerosol Med. 14 (2001) 65–71.
[4] F.A. Knott, N. Southwell, Aerosol penicillin in the oxygen tent, Arch. Dis. Child. 21 (1946) 16–18.
[5] J. Humphrey, H. Joules, Penicillin inhalation in pulmonary disease, Lancet 2 (1946) 221–225.
[6] N. Southwell, Inhaled penicillin in bronchial infections, Lancet 2 (1946) 225–227.
[7] A. Hurst, Penicillin nebulization in bronchopulmonary disease: a preliminary report, Rocky Mt. Med. J. 43 (1946) 219–221.
[8] T. O'Riordan, Inhaled antimicrobial therapy: from cystic fibrosis to the flu, Respir. Care 45 (2000) 836–845.
[9] M.K. McAllen, S.J. Kochanowski, K.M. Shaw, Steroid aerosols in asthma: an assessment of betamethasone valerate and a 12-month study of patients on maintenance treatment, Br. Med. J. 1 (1974) 171–175.
[10] P. Muttil, C. Wang, A.J. Hickey, Inhaled drug delivery for tuberculosis therapy, Pharm. Res. 26 (2009) 2401–2416.
[11] D.E. Geller, P.A. Flume, D. Staab, R. Fischer, J.S. Loutit, D.J. Conrad, f.t.M.S. Group, Levofloxacin inhalation solution (MP-376) in patients with cystic fibrosis with *Pseudomonas aeruginosa*, Am. J. Respir. Crit. Care Med. 183 (2011) 1510–1516.
[12] D.I. Bernstein, P.D. Reuman, J.R. Sherwood, E.C. Young, G.M. Schiff, Ribavirin small-particle-aerosol treatment of influenza B virus infection, Antimicrob. Agents Chemother. 32 (1988) 761–764.
[13] R.A. Mohammad, K.C. Klein, Inhaled amphotericin B for prophylaxis against invasive *Aspergillus* infections, Ann. Pharmacother. 40 (2006) 2148–2154.
[14] M. Aitken, W. Burke, G. McDonald, S. Shak, A. Montgomery, A. Smith, Recombinant human DNase inhalation in normal subjects and patients with cystic fibrosis. A phase 1 study, JAMA 267 (1992) 1947–1951.
[15] L.H. Jones, H. Baldock, M.E. Bunnage, J. Burrows, N. Clarke, M. Coghlan, D. Entwistle, D. Fairman, N. Feeder, C. Fulton, L. Hilton, K. James, R.M. Jones, A.S. Kenyon, S. Marshall, S.D. Newman, R. Osborne, S. Patel, M.D. Selby, E.F. Stuart, M.A. Trevethick, K.N. Wright, D.A. Price, Inhalation by design: dual pharmacology β-2 agonists/M3 antagonists for the treatment of COPD, Bioorg. Med. Chem. Lett. 21 (2011) 2759–2763.
[16] B. de Galan, S. Simsek, C. Tack, R. Heine, Efficacy and safety of inhaled insulin in the treatment of diabetes mellitus, Neth. J. Med. 64 (2006) 319–325.
[17] E. Huland, A. Burger, J. Fleischer, P. Fornara, E. Hatzmann, A. Heidenreich, H. Heinzer, H. Heynemann, L. Hoffmann, R. Hofmann, H. Huland, I. Kampfer, M. Kindler, H. Kirchner, G. Mehlhorn, T.H. Moniak, U. Rebmann, J. Roigas, T.H. Schneider, D. Schnorr, H.J. Schmitz, R. Wenisch, Z. Varga, J. Vinke, Efficacy and safety of inhaled recombinant interleukin-2 in high-risk renal cell cancer patients compared with systemic interleukin-2: an outcome study, Folia Biol. 49 (2003) 183–190.
[18] M. Videira, A.J. Almeida, A. Fabra, Preclinical evaluation of a pulmonary delivered paclitaxel-loaded lipid nanocarrier antitumor effect, Nanomedicine 8 (7) (2012) 1208–1215.
[19] D.A. Hokey, A. Misra, Aerosol vaccines for tuberculosis: a fine line between protection and pathology, Tuberculosis (Edinb.) 91 (2011) 82–85.
[20] D. Lu, L. Garcia-Contreras, P. Muttil, D. Padilla, D. Xu, J. Liu, M. Braunstein, D.N. McMurray, A.J. Hickey, Pulmonary immunization using antigen 85-B polymeric microparticles to boost tuberculosis immunity, AAPS J. 12 (2010) 338–347.
[21] I. Roy, N. Vij, Nanodelivery in airway diseases: challenges and therapeutic applications, Nanomedicine 6 (2010) 237–244.
[22] H.M. Mansour, Y.S. Rhee, X. Wu, Nanomedicine in pulmonary delivery, Int. J. Nanomedicine 4 (2009) 299–319.
[23] M. Bailey, C. Berkland, Nanoparticle formulations in pulmonary drug delivery, Med. Res. Rev. 29 (2009) 196–212.
[24] M. Smola, T. Vandamme, A. Sokolowski, Nanocarriers as pulmonary drug delivery systems to treat and to diagnose respiratory and non respiratory diseases, Int J Nanomedicine 3 (2008) 1–19.
[25] World Health Organization, The top ten causes of death. The 10 leading causes of death by broad income group, http://www.who.int/mediacentre/factsheets/fs310/en 2008.
[26] World Health Organization/The United Nations Children's Fund, Global Action Plan for Prevention and Control of Pneumonia (GAPP), 2009.
[27] A. Zumla, Killer respiratory tract infections: time to turn the tide, Curr. Opin. Pulm. Med. 18 (2012) 173–174.
[28] J. Rello, E. Diaz, Pneumonia in the intensive care unit, Crit. Care Med. 31 (2003) 2544–2551.
[29] World Health Organization, Global Tuberculosis Control: WHO Report 2012, 2012.
[30] R. Rastogi, Y. Sultana, A. Ali, M. Aqil, Particulate and vesicular drug carriers in the management of tuberculosis, Curr. Drug Deliv. 3 (2006) 121–128.
[31] M. Pinheiro, M. Lúcio, J.L. Lima, S. Reis, Liposomes as drug delivery systems for the treatment of TB, Nanomedicine (Lond.) 6 (2011) 1413–1428.
[32] A. Sosnik, A.M. Carcaboso, R.J. Glisoni, M.A. Moretton, D.A. Chiappetta, New old challenges in tuberculosis: potentially effective nanotechnologies in drug delivery, Adv. Drug Deliv. Rev. 62 (2010) 547–559.
[33] R. Pandey, G.K. Khuller, Antitubercular inhaled therapy: opportunities, progress and challenges, J. Antimicrob. Chemother. 55 (2005) 430–435.
[34] D. Pimentel, M. Videira, Lipid nanocarriers as a strategy to promote phagocytosis and tropism for endocytic pathways in macrophages, 1st Post-Graduate iMed.UL Students Meeting, Faculty of Pharmacy, University of Lisbon, Portugal, 2009.
[35] A.H. Limper, K.S. Knox, G.A. Sarosi, N.M. Ampel, J.E. Bennett, A. Catanzaro, S.F. Davies, W.E. Dismukes, C.A. Hage, K.A. Marr, C.H. Mody, J.R. Perfect, D.A. Stevens, A.T.S.F.W. Group, An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients, Am. J. Respir. Crit. Care Med. 183 (2011) 96–128.
[36] R. Laniado-Laborín, M.N. Cabrales-Vargas, Amphotericin B: side effects and toxicity, Rev. Iberoam. Micol. 26 (2009) 223–227.
[37] S.P. Vyas, S. Quraishi, S. Gupta, K.S. Jaganathan, Aerosolized liposome-based delivery of amphotericin B to alveolar macrophages, Int. J. Pharm. 296 (2005) 12–25.
[38] K. Gilani, E. Moazeni, T. Ramezanli, M. Amini, M.R. Fazeli, H. Jamalifar, Development of respirable nanomicelle carriers for delivery of amphotericin B by jet nebulization, J. Pharm. Sci. 100 (2011) 252–259.
[39] A.K. Agrawal, A. Agrawal, A. Pal, P.Y. Guru, C.M. Gupta, Superior chemotherapeutic efficacy of amphotericin B in tuftsin-bearing liposomes against *Leishmania donovani* infection in hamsters, J. Drug Target. 10 (2002) 41–45.
[40] T. Jartti, L. Jartti, O. Ruuskanen, M. Söderlund-Venermo, New respiratory viral infections, Curr. Opin. Pulm. Med. 18 (2012) 271–278.
[41] J.D. Beckham, A. Cadena, J. Lin, P.A. Piedra, W.P. Glezen, S.B. Greenberg, R.L. Atmar, Respiratory viral infections in patients with chronic, obstructive pulmonary disease, J. Infect. 50 (2005) 322–330.
[42] L.A. Rosenthal, P.C. Avila, P.W. Heymann, R.J. Martin, E.K. Miller, N.G. Papadopoulos, R.S. Peebles, J.E. Gern, E.a.O.R.D.I.S., Infections and Asthma Committee, American Academy of Allergy, Asthma & Immunology, Viral respiratory tract infections and asthma: the course ahead, J. Allergy Clin. Immunol. 125 (2010) 1212–1217.
[43] World Health Oraganization, Antimicrobial resistance, http://www.who.int/mediacentre/factsheets/fs194/en/.
[44] Centers for Disease Control and Prevention, Antibiotic/antimicrobial resistance, http://www.cdc.gov/drugresistance/index.html.
[45] L. Zhang, D. Pornpattananangku, C.M. Hu, C.M. Huang, Development of nanoparticles for antimicrobial drug delivery, Curr. Med. Chem. 17 (2010) 585–594.
[46] F. Andrade, M. Videira, D. Ferreira, B. Sarmento, Micelle-based systems for pulmonary drug delivery and targeting, Drug Deliv. Lett. 1 (2011) 171–185.
[47] W.E. Bawarski, E. Chidlowsky, D.J. Bharali, S.A. Mousa, Emerging nanopharmaceuticals, Nanomedicine 4 (2008) 273–282.
[48] R. Duncan, R. Gaspar, Nanomedicine(s) under the microscope, Mol. Pharm. 8 (2011) 2101–2141.
[49] R. Bosetti, L. Vereeck, Future of nanomedicine: obstacles and remedies, Nanomedicine (Lond.) 6 (2011) 747–755.
[50] M. Hamburg, FDA's approach to regulation of products of nanotechnology, Science 336 (2012) 299–300.
[51] U.S. Department of Health and Human Services, Food and Drug Administration, Guidance for Industry: Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology, 2011.
[52] BCC Research, Nanotechnology in medical applications: the global market (HLC069B), http://www.bccresearch.com/report/nanotechnology-medical-applications-global-market-hlc069b.html, (accessed in March 2013).
[53] F. Andrade, M. Videira, D. Ferreira, B. Sarmento, Nanocarriers for pulmonary administration of peptides and therapeutic proteins, Nanomedicine (Lond.) 6 (2011) 123–141.
[54] L. Garcia-Contreras, J. Fiegel, M.J. Telko, K. Elbert, A. Hawi, M. Thomas, J. VerBerkmoes, W.A. Germishuizen, P.B. Fourie, A.J. Hickey, D. Edwards, Inhaled large porous particles of capreomycin for treatment of tuberculosis in a guinea pig model, Antimicrob. Agents Chemother. 51 (2007) 2830–2836.
[55] E. Rytting, J. Nguyen, X. Wang, T. Kissel, Biodegradable polymeric nanocarriers for pulmonary drug delivery, Expert Opin. Drug Deliv. 5 (2008) 629–639.
[56] G. Pilcer, K. Amighi, Formulation strategy and use of excipients in pulmonary drug delivery, Int. J. Pharm. 392 (2010) 1–19.
[57] G. Oberdörster, E. Oberdörster, J. Oberdörster, Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ. Health Perspect. 113 (2005) 823–839.
[58] T.C. Carvalho, J.I. Peters, R.O. Williams, Influence of particle size on regional lung deposition—what evidence is there? Int. J. Pharm. 406 (2011) 1–10.
[59] N.R. Labiris, M.B. Dolovich, Pulmonary drug delivery. Part I: physiological factors affecting therapeutic effectiveness of aerosolized medications, Br. J. Clin. Pharmacol. 56 (2003) 588–599.
[60] M.J. Telko, A.J. Hickey, Dry powder inhaler formulation, Respir. Care 50 (2005) 1209–1227.
[61] M.B. Dolovich, R. Dhand, Aerosol drug delivery: developments in device design and clinical use, Lancet 377 (2011) 1032–1045.
[62] M.J. Donovan, S.H. Kim, V. Raman, H.D. Smyth, Dry powder inhaler device influence on carrier particle performance, J. Pharm. Sci. 101 (2012) 1097–1107.
[63] P.M. Young, D. Cocconi, P. Colombo, R. Bettini, R. Price, D.F. Steele, M.J. Tobyn, Characterization of a surface modified dry powder inhalation carrier prepared by "particle smoothing", J. Pharm. Pharmacol. 54 (2002) 1339–1344.
[64] U.S. Department of Health and Human Services Food and Drug Administration, Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients, 2005.
[65] ICH M3 (R2), Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, 2009.
[66] ICH S3A, Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies, 1994.
[67] M. Zaru, S. Mourtas, P. Klepetsanis, A.M. Fadda, S.G. Antimisiaris, Liposomes for drug delivery to the lungs by nebulization, Eur. J. Pharm. Biopharm. 67 (2007) 655–666.
[68] M.J. de Jesús Valle, R.J. Dinis-Oliveira, F. Carvalho, M.L. Bastos, A. Sánchez Navarro, Toxicological evaluation of lactose and chitosan delivered by inhalation, J. Biomater. Sci. Polym. Ed. 19 (2008) 387–397.
[69] A. Grenha, C. Grainger, L. Dailey, B. Seijo, G. Martin, C. Remuñán-López, B. Forbes, Chitosan nanoparticles are compatible with respiratory epithelial cells *in vitro*, Eur. J. Pharm. Sci. 31 (2007) 73–84.
[70] S. Mura, H. Hillaireau, J. Nicolas, B. Le Droumaguet, C. Gueutin, S. Zanna, N. Tsapis, E. Fattal, Influence of surface charge on the potential toxicity of PLGA nanoparticles towards Calu-3 cells, Int J Nanomedicine 6 (2011) 2591–2605.

[71] M.E. Davis, M.E. Brewster, Cyclodextrin-based pharmaceutics: past, present and future, Nat. Rev. Drug Discov. 3 (2004) 1023–1035.
[72] M.M. Bailey, E.M. Gorman, E.J. Munson, C. Berkland, Pure insulin nanoparticle agglomerates for pulmonary delivery, Langmuir 24 (2008) 13614–13620.
[73] G. Pilcer, F. Vanderbist, K. Amighi, Spray-dried carrier-free dry powder tobramycin formulations with improved dispersion properties, J. Pharm. Sci. 98 (2009) 1463–1475.
[74] N.R. Labiris, M.B. Dolovich, Pulmonary drug delivery. Part II: the role of inhalant delivery devices and drug formulations in therapeutic effectiveness of aerosolized medications, Br. J. Clin. Pharmacol. 56 (2003) 600–612.
[75] B.D. Alexander, T.P. Winkler, S. Shi, E.S. Dodds Ashley, A.J. Hickey, *In vitro* characterization of nebulizer delivery of liposomal amphotericin B aerosols, Pharm. Dev. Technol. 16 (2011) 577–582.
[76] L. Heinemann, The failure of exubera: are we beating a dead horse? J. Diabetes Sci. Technol. 2 (2008) 518–529.
[77] L.B. Kriksunov, A.V. Gumaste, Inhaler, Microdose Technologies Inc., 2008
[78] T.M. Crowder, Highly reproducible powder aerosolisation for lung delivery using powder-specific electromechanical vibration, Expert Opin. Drug Deliv. 2 (2005) 579–585.
[79] K. Wakefield, P.A. Genova, Pneumatic Breath Actuated Inhaler, IEP Pharmaceutical Devices Inc., 2001
[80] H. Pinto-Alphandary, A. Andremont, P. Couvreur, Targeted delivery of antibiotics using liposomes and nanoparticles: research and applications, Int. J. Antimicrob. Agents 13 (2000) 155–168.
[81] E.A. Petersen, J.B. Grayson, E.M. Hersh, R.T. Dorr, S.M. Chiang, M. Oka, R.T. Proffitt, Liposomal amikacin: improved treatment of *Mycobacterium avium* complex infection in the beige mouse model, J. Antimicrob. Chemother. 38 (1996) 819–828.
[82] S. Leitzke, W. Bucke, K. Borner, R. Müller, H. Hahn, S. Ehlers, Rationale for and efficacy of prolonged-interval treatment using liposome-encapsulated amikacin in experimental *Mycobacterium avium* infection, Antimicrob. Agents Chemother. 42 (1998) 459–461.
[83] H. Tomioka, H. Saito, K. Sato, T. Yoneyama, Therapeutic efficacy of liposome-encapsulated kanamycin against *Mycobacterium intracellulare* infection induced in mice, Am. Rev. Respir. Dis. 144 (1991) 575–579.
[84] P.R. Gangadharam, D.R. Ashtekar, D.L. Flasher, N. Düzgüneş, Therapy of *Mycobacterium avium* complex infections in beige mice with streptomycin encapsulated in sterically stabilized liposomes, Antimicrob. Agents Chemother. 39 (1995) 725–730.
[85] M.A. Vladimirsky, G.A. Ladigina, Antibacterial activity of liposome-entrapped streptomycin in mice infected with *Mycobacterium tuberculosis*, Biomed. Pharmacother. 36 (1982) 375–377.
[86] C.E. Swenson, M.C. Popescu, R.S. Ginsberg, Preparation and use of liposomes in the treatment of microbial infections, Crit. Rev. Microbiol. 15 (Suppl. 1) (1988) S1–S31.
[87] C.O. Onyeji, C.H. Nightingale, M.N. Marangos, Enhanced killing of methicillin-resistant *Staphylococcus aureus* in human macrophages by liposome-entrapped vancomycin and teicoplanin, Infection 22 (1994) 338–342.
[88] J. Dhillon, R. Fielding, J. Adler-Moore, R.L. Goodall, D. Mitchison, The activity of low-clearance liposomal amikacin in experimental murine tuberculosis, J. Antimicrob. Chemother. 48 (2001) 869–876.
[89] N. Shaik, G. Pan, W.F. Elmquist, Interactions of pluronic block copolymers on P-gp efflux activity: experience with HIV-1 protease inhibitors, J. Pharm. Sci. 97 (2008) 5421–5433.
[90] S. Suarez, P. O'Hara, M. Kazantseva, C.E. Newcomer, R. Hopfer, D.N. McMurray, A.J. Hickey, Airways delivery of rifampicin microparticles for the treatment of tuberculosis, J. Antimicrob. Chemother. 48 (2001) 431–434.
[91] S. Suarez, P. O'Hara, M. Kazantseva, C.E. Newcomer, R. Hopfer, D.N. McMurray, A.J. Hickey, Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: screening in an infectious disease model, Pharm. Res. 18 (2001) 1315–1319.
[92] S.M. Hwang, D.D. Kim, S.J. Chung, C.K. Shim, Delivery of ofloxacin to the lung and alveolar macrophages via hyaluronan microspheres for the treatment of tuberculosis, J. Control Release 129 (2008) 100–106.
[93] O.R. Justo, A.M. Moraes, Incorporation of antibiotics in liposomes designed for tuberculosis therapy by inhalation, Drug Deliv. 10 (2003) 201–207.
[94] J.M. Abdulla, Y.T. Tan, Y. Darwis, Rehydrated lyophilized rifampicin-loaded mPEG-DSPE formulations for nebulization, AAPS PharmSciTech 11 (2010) 663–671.
[95] N. Changsan, H.K. Chan, F. Separovic, T. Srichana, Physicochemical characterization and stability of rifampicin liposome dry powder formulations for inhalation, J. Pharm. Sci. 98 (2009) 628–639.
[96] M. Zaru, M.L. Manca, A.M. Fadda, S.G. Antimisiaris, Chitosan-coated liposomes for delivery to lungs by nebulisation, Colloids Surf. B Biointerfaces 71 (2009) 88–95.
[97] A. Zahoor, S. Sharma, G. Khuller, Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis, Int. J. Antimicrob. Agents 26 (2005) 298–303.
[98] R. Pandey, G.K. Khuller, Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis, Tuberculosis (Edinb.) 85 (2005) 227–234.
[99] R. Pandey, A. Sharma, A. Zahoor, S. Sharma, G.K. Khuller, B. Prasad, Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis, J. Antimicrob. Chemother. 52 (2003) 981–986.
[100] A. Sharma, S. Sharma, G.K. Khuller, Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis, J. Antimicrob. Chemother. 54 (2004) 761–766.
[101] O.R. Justo, A.M. Moraes, Kanamycin incorporation in lipid vesicles prepared by ethanol injection designed for tuberculosis treatment, J. Pharm. Pharmacol. 57 (2005) 23–30.
[102] T.R. Desai, R.E. Hancock, W.H. Finlay, Delivery of liposomes in dry powder form: aerodynamic dispersion properties, Eur. J. Pharm. Sci. 20 (2003) 459–467.
[103] J.P. Wong, H. Yang, K.L. Blasetti, G. Schnell, J. Conley, L.N. Schofield, Liposome delivery of ciprofloxacin against intracellular *Francisella tularensis* infection, J. Control Release 92 (2003) 265–273.
[104] J.F. Marier, J.L. Brazier, J. Lavigne, M.P. Ducharme, Liposomal tobramycin against pulmonary infections of *Pseudomonas aeruginosa*: a pharmacokinetic and efficacy study following single and multiple intratracheal administrations in rats, J. Antimicrob. Chemother. 52 (2003) 247–252.
[105] J.F. Marier, J. Lavigne, M.P. Ducharme, Pharmacokinetics and efficacies of liposomal and conventional formulations of tobramycin after intratracheal administration in rats with pulmonary *Burkholderia cepacia* infection, Antimicrob. Agents Chemother. 46 (2002) 3776–3781.
[106] A. Omri, Z.E. Suntres, P.N. Shek, Enhanced activity of liposomal polymyxin B against *Pseudomonas aeruginosa* in a rat model of lung infection, Biochem. Pharmacol. 64 (2002) 1407–1413.
[107] M. Alipour, M. Halwani, A. Omri, Z.E. Suntres, Antimicrobial effectiveness of liposomal polymyxin B against resistant Gram-negative bacterial strains, Int. J. Pharm. 355 (2008) 293–298.
[108] K.M. Hindi, A.J. Ditto, M.J. Panzner, D.A. Medvetz, D.S. Han, C.E. Hovis, J.K. Hilliard, J.B. Taylor, Y.H. Yun, C.L. Cannon, W.J. Youngs, The antimicrobial efficacy of sustained release silver-carbene complex-loaded L-tyrosine polyphosphate nanoparticles: characterization, *in vitro* and *in vivo* studies, Biomaterials 30 (2009) 3771–3779.
[109] J.T. McConville, K.A. Overhoff, P. Sinswat, J.M. Vaughn, B.L. Frei, D.S. Burgess, R.L. Talbert, J.I. Peters, K.P. Johnston, R.O. Williams, Targeted high lung concentrations of itraconazole using nebulized dispersions in a murine model, Pharm. Res. 23 (2006) 901–911.
[110] J.M. Vaughn, J.T. McConville, D. Burgess, J.I. Peters, K.P. Johnston, R.L. Talbert, R.O. Williams, Single dose and multiple dose studies of itraconazole nanoparticles, Eur. J. Pharm. Biopharm. 63 (2006) 95–102.
[111] C.A. Alvarez, N.P. Wiederhold, J.T. McConville, J.I. Peters, L.K. Najvar, J.R. Graybill, J.J. Coalson, R.L. Talbert, D.S. Burgess, R. Bocanegra, K.P. Johnston, R.O. Williams, Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis, J. Infect. 55 (2007) 68–74.
[112] B.J. Hoeben, D.S. Burgess, J.T. McConville, L.K. Najvar, R.L. Talbert, J.I. Peters, N.P. Wiederhold, B.L. Frei, J.R. Graybill, R. Bocanegra, K.A. Overhoff, P. Sinswat, K.P. Johnston, R.O. Williams, *In vivo* efficacy of aerosolized nanostructured itraconazole formulations for prevention of invasive pulmonary aspergillosis, Antimicrob. Agents Chemother. 50 (2006) 1552–1554.
[113] J.M. Vaughn, N.P. Wiederhold, J.T. McConville, J.J. Coalson, R.L. Talbert, D.S. Burgess, K.P. Johnston, R.O. Williams, J.I. Peters, Murine airway histology and intracellular uptake of inhaled amorphous itraconazole, Int. J. Pharm. 338 (2007) 219–224.
[114] W. Yang, J. Tam, D.A. Miller, J. Zhou, J.T. McConville, K.P. Johnston, R.O. Williams, High bioavailability from nebulized itraconazole nanoparticle dispersions with biocompatible stabilizers, Int. J. Pharm. 361 (2008) 177–188.
[115] R.O. Williams III, R.O. Zimmerer, J.T. MxConville, J.A. Tolman, N.P. Wiederhold, J.I. Peters, Treatment of Pulmonary Fungal Infection with Voriconazole via Inhalation, Board of Regents, The University of Texas System, CyDex Pharmaceuticals, Inc., 2009
[116] B.E. Ragle, V.A. Karginov, J. Bubeck Wardenburg, Prevention and treatment of *Staphylococcus aureus* pneumonia with a beta-cyclodextrin derivative, Antimicrob. Agents Chemother. 54 (2010) 298–304.
[117] S.P. Vyas, M.E. Kannan, S. Jain, V. Mishra, P. Singh, Design of liposomal aerosols for improved delivery of rifampicin to alveolar macrophages, Int. J. Pharm. 269 (2004) 37–49.
[118] F. Ahsan, I.P. Rivas, M.A. Khan, A.I. Torres Suarez, Targeting to macrophages: role of physicochemical properties of particulate carriers–liposomes and microspheres–on the phagocytosis by macrophages, J. Control Release 79 (2002) 29–40.
[119] S. Chono, T. Tanino, T. Seki, K. Morimoto, Efficient drug targeting to rat alveolar macrophages by pulmonary administration of ciprofloxacin incorporated into mannosylated liposomes for treatment of respiratory intracellular parasitic infections, J. Control Release 127 (2008) 50–58.
[120] S.P. Vyas, K. Khatri, Liposome-based drug delivery to alveolar macrophages, Expert Opin. Drug Deliv. 4 (2007) 95–99.
[121] M.A. Moretton, D.A. Chiappetta, F. Andrade, J. das Neves, D. Ferreira, B. Sarmento, A. Sosnik, Hydrolyzed galactomannan-modified nanoparticles and flower-like polymeric micelles for the active targeting of rifampicin to macrophages, J. Biomed. Nanotechnol. 9 (2013) 1–12.
[122] M. Zaru, C. Sinico, A. De Logu, C. Caddeo, F. Lai, M.L. Manca, A.M. Fadda, Rifampicin-loaded liposomes for the passive targeting to alveolar macrophages: *in vitro* and *in vivo* evaluation, J. Liposome Res. 19 (2009) 68–76.
[123] K. Ohashi, T. Kabasawa, T. Ozeki, H. Okada, One-step preparation of rifampicin/poly(lactic-co-glycolic acid) nanoparticle-containing mannitol microspheres using a four-fluid nozzle spray drier for inhalation therapy of tuberculosis, J. Control Release 135 (2009) 19–24.
[124] C.M. Gupta, W. Haq, Tuftsin-bearing liposomes as antibiotic carriers in treatment of macrophage infections, Methods Enzymol. 391 (2005) 291–304.
[125] A. Agarwal, H. Kandpal, H.P. Gupta, N.B. Singh, C.M. Gupta, Tuftsin-bearing liposomes as rifampin vehicles in treatment of tuberculosis in mice, Antimicrob. Agents Chemother. 38 (1994) 588–593.
[126] M. Owais, I. Ahmed, B. Krishnakumar, R.K. Jain, B.K. Bachhawat, C.M. Gupta, Tuftsin-bearing liposomes as drug vehicles in the treatment of experimental aspergillosis, FEBS Lett. 326 (1993) 56–58.
[127] L. Sweeney, Z. Wang, R. Loebenberg, J. Wong, C. Lange, W. Finlay, Spray-freeze-dried liposomal ciprofloxacin powder for inhaled aerosol drug delivery, Int. J. Pharm. 305 (2005) 180–185.
[128] F. Tewes, J. Brillault, W. Couet, J.C. Olivier, Formulation of rifampicin–cyclodextrin complexes for lung nebulization, J. Control Release 129 (2008) 93–99.