Inhalable nanoparticulate powders for respiratory delivery

Priya Muralidharan, Monica Malapita, Evan Mallory, Don Hayes Jr., Heidi M. Mansour

The University of Arizona, College of Pharmacy, Skaggs Pharmaceutical Sciences Center, Tucson, AZ, USA

The Ohio State University College of Medicine, Departments of Pediatrics and Internal Medicine, Lung and Heart-Lung Transplant Programs, Columbus, OH, USA

The Ohio State University College of Medicine, The Davis Heart and Lung Research Institute, Columbus, OH, USA

The University of Arizona, The BIO5 Research Institute, Tucson, AZ, USA

The University of Arizona, Institute of the Environment, Tucson, AZ, USA

The University of Arizona, National Cancer Institute Comprehensive Cancer Center, Tucson, AZ, USA

Received 8 September 2014; accepted 15 January 2015

Abstract

Nanoparticles are extensively studied for drug delivery and are proving to be effective in drug delivery and the diagnostic field. Drug delivery to lungs has its advantages over other routes of administration. Inhalable powders consisting of nanoparticles are gaining much interest in respiratory research and clinical therapy. Particle engineering technique is a key factor to develop inhalable formulations that can successfully deliver drug with improved therapeutic effect and enhanced targeting. Inhalable nanoparticles in the solid-state dry powders for targeted pulmonary delivery offer unique advantages and are an exciting new area of research. Nasal delivery of inhalable nanoparticulate powders is gaining research attention recently, particularly in vaccine applications, systemic drug delivery in the treatment of pain, and non-invasive brain targeting. Fundamental aspects and recent advancements along with future prospects of inhalable powders consisting of nanoparticles in the solid-state for respiratory delivery are presented.

From the Clinical Editor: The advance in nanotechnology has enabled the design of new drug delivery systems through inhalation, which has many advantages over traditional delivery systems. This comprehensive review describes and discusses the current status, drug design and modification for targeted delivery and challenges of the use of nanoparticles in the respiratory tract.

© 2015 Elsevier Inc. All rights reserved.

Key words: Pulmonary nanomedicine; Solid-state; Dry powder inhaler; Particle engineering design; Inhalation powders; Lung/nasal

Systemic delivery of drugs through inhalation (oral and nasal) is an attractive alternative for oral or parenteral drug delivery. Drug delivery to lungs through inhalation has advantages such as high bioavailability, rapid onset of action due to its large surface area for absorption, improved patient compliance, non-invasive nature, limited drug degradation, and high solute permeability. Pulmonary route has been used for local delivery of drugs like antibiotic, protein, peptide, chemotherapeutics, interferon, antitrypsin, protease inhibitors, deoxyribonucleases, vaccines and many more. An important consideration in pulmonary delivery is aerosolization of the drug. Delivery of drug to the lungs has to go through physical obstruction and physiological obstruction which includes the multiple bifurcation of respiratory tract and the innate immunological response. However, inhalation is not new, inhaled fumigation was known in the first century and antiseptic aerosol therapy was popular in mid-20th century. Particles deposit in the respiratory track by virtue of their size, shape and surface properties. There are three main mechanisms by which particles deposit in respiratory tract: impaction, sedimentation and/or diffusion. Particles deposit in the mid and deep lung regions when the aerodynamic particle size is ≤5 μm, which is where nanoparticles have a niche in advanced pulmonary drug delivery. Nanoparticles can be used for targeted delivery, sustained delivery and deep lung delivery of drugs and therapeutics. A recent term, “nanoperiodic property”, has been introduced by Kannan et al, which relates nanoparticle behavior to its in vivo behavior. Particle size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition have been identified as “critical nanoscale...
design parameters (CNDP) which can be used to control and engineer particles to optimize pharmacokinetics, pharmacodynamics and site-specific disease targeting. Nanotechnology is currently revolutionizing drug delivery especially in inhalation drug delivery. This review discusses the use of nanoparticles as dry powders for respiratory delivery of drugs.

Inhalable powders for lung delivery

Particle deposition in the lung depends predominantly on its properties including particle size, size distribution, particle morphology, surface morphology, hygroscopicity, electrical charge and density. Other factors include the diseased state and breathing pattern. The geometric diameter of a particle is less influential than aerodynamic diameter. Hence, the United States Pharmacopeia (USP) Chapter defines mass median aerodynamic diameter (MMAD). MMAD means that 50% of particles in the aerodynamic size distribution, based on mass, lie above and below that diameter. Larger particles deposit in the airway due to inertia impaction and sedimentation while smaller particles deposit by diffusion.

Ciliated columnar epithelium in the upper airway secretes mucus which is a thick gel layer. The primary function of the mucosal layer is to protect the lungs by trapping and removing foreign particles by the mucociliary escalator which causes trapped particles to be coughed up out of the lungs. Particles reaching the deep lung alveolar region may be susceptible to clearance by alveolar macrophages by phagocytosis depending on the surface chemistry of the particles. To evade mucociliary trapping and clearance, the inhaled particle should either be of small size to be inhaled past the upper lung region or have the appropriate surface chemistry to avoid adhesion to the mucosal layer and/or mucocpentration. Use of hydrophilic and neutrally charged polymers helps in escaping mucus adhesion. Lung phagocytosis can be significant for particles of geometric diameter (dg) $\mu m \leq dg \leq 2 \mu m$, dependent on the surface chemistry of the particles, and decreases for particles smaller and larger. Dense surface charge and low molecular weight PEGylated nanoparticles can penetrate the mucus.

Infected airways have compromised mucociliary clearance and are vulnerable to bacterial biofilm formation, which is highly resistant to antibiotics and requires additional dose through conventional routes of administration. Inhalation of antibiotic for pulmonary infection has been proven clinically to be more effective than other routes of administration. Nanoparticles in the size range of 200 nm are effective in mucus penetration. Creating nanoparticles to exhibit biphasic release profile will give high initial burst followed by sustained release of antibiotic to maintain sufficient drug concentration to inhibit biofilm growth. Additionally PEGylated liposomal formulations have proved to be effective in mucus penetration and escaping pulmonary and immune clearance.

Nasal delivery of nanoparticles

Nasal route is a choice for vaccine delivery due to ease of delivery through nose, high vascularity in nose, large surface area for absorption and low enzymatic degradation. Inhalable powder formulations for nasal delivery enhance systemic bioavailability and are superior to liquid formulations. Advantages of dry powders also include increased chemical stability, no requirement for preservatives, and feasibility of administering relatively large amounts of drug. Improved nasal delivery of vaccines through nanoparticles may be effective at promoting improved uptake of particles by the nasal-associated lymphoid tissue (NALT) system. Nanoparticles larger than 20 nm will cross mucosal membranes through the transmucosal route using endocytosis, carrier-mediated or receptor-mediated transport processes. There is no significant difference in immune response between nano and microparticles.

Mucoadhesion is key to nasal delivery of drugs. Chen et al formulated liposomes of bovine serum albumin coated with polymer to increase bioavailability and mucoadhesion. The liposomes were made of soy phosphatidylcholine (SPC) and phospholipid dimyristoyl phosphatidylglycerol (DMPG) coated with alginate, chitosan or trimethyl chitosan (TMC). Polymer coating resulted in increased size of liposome. However, mucoadhesion property of chitosan and TMC particles increased compared to alginate coated and uncoated particles. Dehghan et al formulated a polymeric nanosphere nasal vaccine for influenza which enters the body through the inhalation route. In the study, they prepared dry nanoparticle powders of influenza vaccines with two other immunoadjuvants using chitosan as the carrier. The formulation demonstrated that the vaccine structure and characteristics of chitosan did not change after the formulation. The particles had a size of 581.1 ± 32.6 nm with mucoadhesive properties of chitosan that makes it suitable for nasal delivery of vaccine. Dry powder chitosan nanospheres may be an appropriate delivery system for nasal immunization of influenza, due to the nano size range, the ability for chitosan to adhere to mucosal membranes, and suitable release profile. Another study on nasal vaccine delivery was conducted by Wang et al where they formulated anthrax vaccine for dry powder nasal delivery. Vaccination at the site of entry can be more effective than the systemic route, simply because the pathogens can be encountered and neutralized at entry before it gets into the systemic circulation. The nasal route is preferred for its mucous layer, hence nasal products should be mucoadhesive. Inhalable nasal powders are gaining popularity as new vaccine delivery by virtue of their stability compared to liquid formulations that require refrigeration or preservatives. A report by Wang et al investigated a nasal formulation composed of recombinant protective antigen, compound 48/80 mast cell activator as a mucosal adjuvant, and trehalose. The particle size was ~25 μm and the vaccine maintained its structural integrity throughout processing. In vivo study of the formulation, in rabbits showed the vaccines competence to neutralize anthrax lethal toxin. They also found that the dry powder vaccine was effective even after 2.5 years of storage at room temperature which will alleviate the cold chain shipping problem for vaccine. An in-situ gel forming dry powder formulation was developed by Velasquez et al using norovirus like particles with mucoadhesive polymer GelSite. In vivo study of the formulation showed that the vaccine induced higher antigen response than liquid preparation.
Dry powder inhalers and nanoparticulate powders for inhalation

The overall anatomy and physiology of the pulmonary system are complicated and the dynamic pulmonary clearance mechanisms present challenges for drug delivery through this route. Despite these potential challenges, there are four clinically successful pulmonary inhalation pharmaceutical dosage forms based on device classes; namely, nebulizers (nebs), pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs), and soft-mist inhalers (SMIs). Nebulizers produce liquid aerosols by an external power supply and do not contain any propellant unlike pMDIs. Nebulizers require an external power source and owing to its size is restricted to clinical settings and in-house use for niche patient populations (i.e. young children and the elderly), while pMDI offers portability and patient convenience. However, propellant effects on the environment, solubility and compatibility challenges of drug with propellant and physicochemical stability challenges are common. DPIs can contain respirable powdered drug or respirable powdered drug blended with a non-respirable carrier. There are many unique advantages of DPIs. Powdered drug offers an advantage especially for delivery of poorly water-soluble drug, and protein and peptide drugs which cannot withstand the shear generated during inhalation. However, the choice of non-respirable carrier is critical for DPI formulation. Lactose is the carrier of choice owing to its historical precedence, large supply, and FDA approval for DPI use. However, patient lactose intolerance, patient lactose allergies, and reducing sugar chemical property leading to chemical degradation issues by the Maillard reaction with certain pulmonary drugs are limitations of the lactose carrier. Hence, other non-respirable carriers have been studied (some of which are approved outside the United States) including non-reducing sugars (e.g. mannitol and trehalose), glucose, sodium chloride, erythritol, sorbitol, raffinose, xylitol, dextrose, maltitol and maltose as potential carriers that can be used for DPI.

Afrezza®, which is inhalable recombinant insulin contains Technosphere® particles formed with excipient carrier fumaryl diketopiperazine (FDKP) powder which self assembles through hydrogen bonding in mildly acidic environment to form microspheres. DPIs offer many advantages including encapsulating ability, long term stability, no hand-lung inhalation coordination, no liquid propellant, modified pharmacokinetics, an extended release profile, improved tolerability, reduced toxicity, easy to use, and noninvasiveness. Based on the mechanisms of particle dispersion and aerosolization, the DPI devices are further categorized as passive or active devices. A passive DPI device depends on the patient’s inspiratory flow to supply the energy required for powder dispersion. Variation in patient’s inspiratory flow can vary the quantity of drug delivered which might lead to overdosing or underdosing. In contrast, an active DPI device does not depend on a patient’s inspiratory flow. The DPI device that was used in Exubera® was the first active device used in a FDA-approved pharmaceutical inhalation product. However, the product didn’t last long in the market due to other reasons. Depending on the drug dispensing method DPI devices are classified into three types namely unit dose, multidose (i.e. powder reservoir), and multi-unit dose DPI devices. A unit dose DPI device requires the patient to insert an inhalation grade capsule (i.e. gelatin or hydroxypropylmethylcellulose) containing the preweighed drug powder prior to each actuation. Upon actuation, the capsule breaks apart or is pin-holed by the device and releases the powder for aerosolization. Multi dose reservoir device contains a powder bed of drug or drug/lactose monohydrate blend which is sampled by the device metering system with each actuation by patient. A multi-unit dose DPI device is pre-loaded with multiple unit dose prefilled capsules containing powder.

DPI is a rapidly growing sector of the pulmonary inhalation pharmaceutical market which is evident by the increasing number of successful products in the market. Recent FDA approval of Afrezza®, the inhaled insulin will invite more research and growth into inhalation therapy. DPIs can have two potential problems concerning relatively low fine particle fraction (FPF) and emitted dose (ED) which can be attributed to insufficient particle dispersion by the patient or DPI device, aerosol dispersion inefficiency, or the powder formulation itself. FPF is the fraction of inhaled particles that are smaller than a certain aerodynamic diameter, and ED is the proportion of initial dose that is delivered out of the device, as described in USP Chapter <601>. The emerging technologies in overcoming these problems will be discussed in detail in this article.

The improved formulations have made it possible to deliver small (micro/nano size) particles to the lungs, yet due to low inertia these particles can be exhaled from lungs and fail to deposit in the lungs. Hence, to enable better delivery they are usually formulated with a large non-respirable carrier which adds bulk to the powder and helps in efficient metering of the dose. On actuation, the drug particles along with the carrier gets dispersed into patients mouth but only the respirable drug particles will reach the respiratory tract. The large non-respirable carrier particle separates from the drug particle by shear or mechanical forces and it gets deposited onto the oropharynx and is swallowed in to the gastrointestinal tract. The separation of these particles depends on the interparticulate forces.

The interparticulate interfacial interactions that impact DPI aerosol dispersion are van der Waals forces, electrostatic forces, and capillary forces. These forces are important for aerosolization of powder from the device during delivery and separation of drug from the carrier particles. Inter particular forces vary with materials used and the way it is processed. Interparticulate forces can be altered by the particle size, particle size distribution, surface morphology (i.e. surface roughness), particle shape, elastic/plastic deformity, drug/carbon ratio and drug/fine ratio. Xu et al reviewed particle interactions in dry powder inhaler in detail, the article is recommended for further reading.

The differences between microparticles and nanoparticles extend beyond just the size. Nanoparticles can have higher drug loading capacity, use less polymers, can better cross permeability barriers, increased cellular uptake, longer lung retention and in airway nanoparticles have better chances of mucus penetration. Nanoparticles in general have larger surface area to volume ratios. This improves dissolution properties wherein decreased particle size increases solubility and intracellular drug delivery potential. Owing to smaller size, nanoparticles present more molecules on the surface of the particle thus increasing the total mass that may transfer to the surrounding medium. This property can be used to achieve increased drug concentration and bioavailability. Studies have demonstrated...
that particles with decreased size are better internalized by cells.\textsuperscript{37} Nanoparticles can act as drug carriers by dissolving, entrapping, encapsulating, adsorbing, or attaching to the drug.\textsuperscript{2}

Nanoparticles are used in both dry powder inhaler and nasal delivery of therapeutics. Table 1 lists some of the drugs which have been successfully made into inhalable nanofluids for targeted respiratory delivery. However, further details on their successful use in rodents or human stands to be explored. In targeted respiratory delivery. However, further details on their successful use in rodents or human stands to be explored. In vivo study conducted in a rabbit model

### Table 1
Examples of drugs made into nanoformulations as dry powder inhalers (DPIs).

<table>
<thead>
<tr>
<th>Drug/agent</th>
<th>Class</th>
<th>Condition</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>Antibiotic</td>
<td>Infection</td>
<td>DPI\textsuperscript{57}</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>Antibiotic</td>
<td>Infection</td>
<td>DPI\textsuperscript{57}</td>
</tr>
<tr>
<td>Salmon calcitonin</td>
<td>Hormone</td>
<td>Hypocalcemia</td>
<td>DPI\textsuperscript{85}</td>
</tr>
<tr>
<td>Tacrolimus and cyclosporine A</td>
<td>Immunosuppressant</td>
<td>Allograft rejection prevention in lung transplantation</td>
<td>DPI\textsuperscript{7,10}</td>
</tr>
<tr>
<td>Budesonide</td>
<td>Glucocorticoid</td>
<td>Asthma and COPD</td>
<td>DPI\textsuperscript{74*}</td>
</tr>
<tr>
<td>Tranilast</td>
<td>Anti-allergic agent</td>
<td>Bronchial asthma</td>
<td>DPI\textsuperscript{73}</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>Radio contrast agent</td>
<td>Imaging of airway</td>
<td>DPI\textsuperscript{66*}</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Antibiotic</td>
<td>Cystic fibrosis</td>
<td>DPI\textsuperscript{62}</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>Immunosuppressant</td>
<td>Lung transplant rejection prevention</td>
<td>DPI\textsuperscript{7,87*}</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Microtubule inhibitor</td>
<td>Lung cancer</td>
<td>DPI\textsuperscript{58}</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>Antibiotic</td>
<td>Infection</td>
<td>DPI\textsuperscript{56}</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Antibiotic</td>
<td>Infection</td>
<td>DPI\textsuperscript{56}</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Antibiotic</td>
<td>Tuberculosis</td>
<td>DPI\textsuperscript{58}</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Antibiotic</td>
<td>Infection</td>
<td>DPI\textsuperscript{69}</td>
</tr>
<tr>
<td>Mexofloxacin</td>
<td>Antibacterial</td>
<td>Infection</td>
<td>DPI\textsuperscript{69}</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Anticancer agent</td>
<td>Lung cancer</td>
<td>DPI\textsuperscript{90}</td>
</tr>
<tr>
<td>Influenza</td>
<td>Antigen</td>
<td>Influenza</td>
<td>Nasal\textsuperscript{27}</td>
</tr>
<tr>
<td>Anthrax rPA</td>
<td>Antigen</td>
<td>Anthrax</td>
<td>Nasal\textsuperscript{38*}</td>
</tr>
<tr>
<td>Fluticasone propionate/ albuterol sulfate</td>
<td>Anti-inflammatory/ β2-agonist</td>
<td>Asthma and COPD</td>
<td>DPI\textsuperscript{91}</td>
</tr>
<tr>
<td>Salbutamol sulfate</td>
<td>β2-agonist</td>
<td>Asthma</td>
<td>DPI\textsuperscript{92}</td>
</tr>
</tbody>
</table>

Abbreviation: COPD – chronic obstructive pulmonary disease; rPA – recombinant protective antigen.

* In vivo study conducted in a rat model

** In vivo study conducted in a rabbit model

Modification of inhalable nanoparticles

The most common challenges in using nanoparticles for DPI delivery are i) maintaining the particle in dry state until delivery, ii) to prevent aggregation of the particles in the inhaler, iii) efficient redispersion of drug in the lung fluid, iv) preservation of the particle and the biological activity of the drug throughout processing stages. Particle engineering is a convenient tool to achieve particles of desired characteristics with lesser expense.\textsuperscript{44} Various techniques can be adopted to make particles with narrow size distribution.\textsuperscript{44} Improved dispersibility,\textsuperscript{44} sustained release\textsuperscript{44} with inhalable properties. Some popular particle engineering techniques are i) surface modification to improve nanoparticle characteristics as a delivery vehicle and to protect it from deterioration, ii) making large hollow particle for deep lung deposition, iii) encapsulating nanoparticles within microparticles to prevent particle aggregates, iv) making effervescent particles to improve dispersion. Table 2 lists some techniques that can be used to fabricate particles for inhalation.

### Surface modification

Surface coating of nanoparticles with neutrally charged molecules such as poly (ethylene glycol) (PEG) has demonstrated many advantages which includes improved transport of particles across mucous layer.\textsuperscript{4} This would enhance the chances of the particle survival that could reach deep lung sites. It is also showed by several studies that PEGylation of nanoparticles evades phagocytosis by alveolar macrophage and improved bioavailability of the drug.\textsuperscript{45-50} The steric hindrance and the negative zeta potential created by PEG molecule help it escape from blood protein.\textsuperscript{46}

Once the particles are modified to reach the lung, translocation across air-blood barrier is the next obstacle.\textsuperscript{1} Smaller molecules that make it to the lungs are cleared quickly while larger particles like protein are degraded by protease enzymes. Hence, it is necessary to encapsulate the drug molecule into nanoparticle to avoid pulmonary clearance or degradation and ensure sustained release.\textsuperscript{1} Most popular encapsulation is done in polymer or liposome. Interestingly, surface modification also has an effect in translocation of nanoparticles. Neutral and negatively charged particles were more rapidly translocated than cationic particles.\textsuperscript{1,4}

### Hollow nanoparticles and nanoaggregates

Formulating nanoparticles into large hollow or porous particles increases the geometric diameter and decreases aerodynamic diameter of particles, thereby making the particle more suitable to deposit in the lung.\textsuperscript{18} Geometric diameter of a particle contributes less to particle deposition while aerodynamic diameter determines the deep lung deposition of nanoparticles. Large particles can be improved to behave like small particles. A study conducted by Edward et al demonstrated that porous particles with drug have a higher aerosolization efficiency, sustained release and increased bioavailability.\textsuperscript{51}

Nanoaggregate are drug containing nanoparticles accumulated together, which may dissociate into individual nanoparticle and release the drug in the lungs or respiratory tract. Large hollow nanoparticulate aggregates which possess geometric diameter ~10 μm exhibit a small aerodynamic
diameter (1-5 μm) due to low particle density. The larger particles reduce the tendency for the particles to aggregate in the inhaler device which will ensure proper delivery of the powder while the smaller particle will avoid deposition elsewhere in the respiratory tract but the lungs.

**Liposomal nanoparticles**

Nanoparticles with phospholipids on the surface escape opsonin attack, which is responsible for phagocytosis and it also significantly improves the fine particle fraction. In addition, liposomal nanoparticles can be produced in different size ranges that can be used for targeted drug delivery, these particles can efficiently encapsulate variety of drugs. Additionally liposomal surface can be modified with polymer to improve its circulating properties and by using lipids indigenous to lung liposomal nanoparticles will be well tolerated. Hadinoto et al prepared a phospholipid nanoparticle aggregate made of polycrylate and silica nanoparticles. They found that the degree of hollowness of the nanoparticles varies according to the phospholipid concentration. It also depends on the chemical nature and size of the nanoparticles. However, phospholipid does not affect the morphology or physical state of the particles. A DPI formulation of dapsone encapsulated in nano-liposomes offers promise for the treatment of *Pneumocystis carinii* pneumonia (PCP) by avoiding systemic side effects and achieving higher drug concentrations at the site of infection.

**Nano-in-micro and polymeric nanoparticles**

To improve dispersion and deep lung deposition of nanoparticle, modified micro particle carriers are used. This formulation reduces particle–particle interaction in addition to improved handling and delivery of nanoparticles. Figures 1 and 2 illustrates the dispersion mechanisms of inhalable nanoparticles as inhaled dry powders with and without large non-respirable carrier particles (i.e. “carrier-free”). A study demonstrated the potential for pulmonary DPI delivery of human IgG utilizing a simultaneously manufactured nano-in-micro (SIMANIM) particle. This one-step spray drying process allowed for deep lung penetration and approximately 35 days of release of IgG. The results of this study could have a wide range of implications in the utilization of antibodies to treat respiratory pathogens.

As discussed earlier in surface modification, addition of some polymers to the surface of nanoparticles to make it hydrophilic can render it mucopenetrating or capable of escaping opsonin attack. Materials used for the purpose include poly(ethylene glycol) (PEG), methoxy poly(ethylene glycol) (MPEG), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) and vitamin E d-a-succinatated polyethylene glycol 1000 (vitamin E TPGS). Nanoparticles can be encapsulated in polymer based carriers, loaded on the surface of the polymer or dispersed in polymeric matrix. Polyelectrolyte complexes use oppositely charged polymers to entrap drugs into a polymeric matrix nanoparticle, which then releases the drug either through polymer degradation or drug diffusion. The matrix of the carrier particle can consist of only nanoparticles or additional inert pharmaceutical excipients, such as amino acids, sugars or phospholipids. Upon deposition in the lungs and exposure to the humid environment and the lung lining fluid, the polymer matrix dissolves and readily releases the nanoparticles. Nanostructures (Figure 3) are made by binding the nanoparticles aggregates with an excipient, mostly polymer. Poly(DL-lactide-co-glycolide acid) (PLGA) is an FDA approved, most popular, polymer used for nanoparticle delivery due to its safety profile, controlled release properties and improved colloidal stability. Yang et al successfully formulated a salmon calcitonin adsorbed polymeric PLGA nanosphere which was coated onto a lactose carrier to form nanocomposite. The nanocomposite particles had efficient lung deposition and rapid release of salmon calcitonin occurred. Another interesting aspect of polymeric nanoparticle was explored by Wang et al who successfully studied various use of nanoparticles to deliver hydrophilic and hydrophobic chemotherapeutics in the same nanoparticles, they also succeeded in formulating multidrug nanoparticle for co-delivery to treat cancer.

Lipid–polymer nanoparticle is a hybrid delivery system, where the polymer nanoparticle core is enveloped in a liposomal layer. It possesses combined properties of polymer and liposomal drug delivery. Yang et al attempted to utilize a new
approach of electrostatically-driven assembly of nanoparticles in developing a dry powder formulation.\textsuperscript{59} In the study, cationic and anionic poly (lactic-co-glycolic acid)/phosphatidylcholine (PLGA/PC) lipid–polymer hybrid nanoparticles were adsorbed on the surface of chitosan carrier particles. The cationic PLGA/PC with stearylamine adsorbed on the surface of negatively charged chitosan carrier particles by electrostatic interaction. However, the formulation had a low loading capacity (18%). The nanoparticle adsorption onto the carrier particle is dependent on the individual charges of all components of carrier.\textsuperscript{59} This can be exploited in the future to modify the carrier surface to accommodate both cationic and anionic nanoparticle adsorption. The formulation had poor desorption characteristics which may lead to low dispersibility in the lung that can reduce the therapeutic dose of the drug.\textsuperscript{59}

**Effervescent nanoparticles**

Effervescent technology has been very popular with oral drug delivery, the same technology is extended to DPI to improve particle dispersion. Several studies have been conducted on the potential use of effervescent technology in carrier particle for dry powder inhaler.\textsuperscript{42,60-62} Some of those studies are discussed in this paper. Effervescent technology can impart an active release mechanism of drug from the formulation, thereby achieving a faster action. In effervescent technology sodium carbonate and citric acid are added to the formulation, which forms gas bubbles when it comes in contact with water hence increasing the volume during the phase transition from solid to gas.\textsuperscript{2} Effervescent carrier formulation generally includes sodium bicarbonate, ammonium hydroxide, and citric acid. Ammonia, ammonium
salt or a suitable buffer is added to maintain pH that would prevent the particles from effervescing during the processing of the powders. In a study conducted by Roa et al inhalable effervescent nanoparticles containing doxorubicin were compared to non-effervescent nanoparticle, nanoparticle solution and suspension injected intravenously. The group of animals treated with effervescent nanoparticles was the only ones that survived the cancer. The effervescent reaction of the carrier particle prevented agglomeration of the nanoparticles and improved their dispersion. In another study, Al-Hallak et al observed the distribution of effervescent nanoparticles containing doxorubicin after pulmonary delivery. The nanoparticle carried in effervescent microparticle achieved deep lung deposition followed by release of doxorubicin. The nanoparticle deposition was primarily found in the lung with little extra pulmonary deposition, however, no drug was found in other organs or tissues. The nanoparticles had long retention and wide distribution in the lung, which can be ascribed to the release of nanoparticle from the effervescent carrier. A comparative study was conducted by Ely et al on drug release and dispersion of ciprofloxacin nanoparticles between effervescent carrier and lactose carrier. The effervescent carrier particles released almost twice as much drug as conventional lactose particle. Effervescent reaction generates force that helps nanoparticles to disperse and avoid aggregation. Thus, effervescence technology has improved release features compared to those that will only dissolve. A pilot safety study was conducted by Azarmi et al on effervescent nanoparticle delivery and showed no negative impact on treated mice population. This is a good starting point for further safety testing of effervescent particles for pulmonary delivery.

**Engineering of nanoparticulate powders for inhalation as DPIs**

Method of preparation of nanoparticle is vital for its performance. There are several methods for the preparation of pulmonary nanoparticles. Some common methods used in the preparation of powders for inhalation will be discussed in this paper as described in Table 3. Generally nanoparticles are prepared by two ways namely: i) precipitation of nanoparticles out of solution (bottom-up) ii) milling larger particles to reduce size (top-down).

### Table 3

<table>
<thead>
<tr>
<th>Methods of particle preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milling</td>
</tr>
<tr>
<td>High-pressure homogenization</td>
</tr>
<tr>
<td>Advanced spray drying</td>
</tr>
<tr>
<td>Spray freeze drying</td>
</tr>
<tr>
<td>Supercritical fluid (antisolvent) extraction</td>
</tr>
<tr>
<td>Inverse phase nanoprecipitation</td>
</tr>
<tr>
<td>Particle replication in non-wetting template (PRINT)</td>
</tr>
<tr>
<td>Controlled aerosol growth</td>
</tr>
<tr>
<td>Thermal condensation aerosols</td>
</tr>
</tbody>
</table>

Spray drying is the most commonly used high throughput method to prepare dry powders for inhalation. In spray drying the drug solution or suspension is introduced at high pressure through the nozzle (atomizing) with spray-air into a heated chamber where the solvent evaporates and the solid dries out followed by separation of the particles using a cyclone separator. The spray drying process has been successfully used to make nanoparticles with smooth surface, narrow particle size distribution, reduced residual water content, nano liposomes, nano-hollow particles and nanocomposites. Spray drying has been used to develop respirable tacrolimus dry powders for targeted deep lung delivery by Wu et al. They employed the process of organic solution advanced spray drying in a closed mode. They successfully produced particles with spherical shapes and smooth surfaces with decreased residual water. Use of an organic solution in spray drying will assist in achieving lower water content in the final product. Spray drying can be used to engineer particles with respect to particle size, shape, density and moisture content. The types of particles and particle properties produced after the spray drying process depend upon a plethora of parameters including solvent used, solute concentration, inlet temperature, outlet temperature, atomizing pressure, feed properties, pump rate, gas type and gas flow rate. Studies have been conducted on the pump rate of spray drying and its influence on particle character. Meenach et al found that higher pump rates of 30 mL/minute reduced the outlet temperature and resulted in smooth and spherical phospholipid/lipopolymeric particles.
This was further confirmed by Li et al who studied characteristics of two antibiotics (tobramycin and azithromycin) by spray drying. They found that the physicochemical property of the drug played a major role in the characteristics of the spray dried particle. Tobramycin with enhanced hygroscopicity had higher residual water content than azithromycin which had lower residual water content. In another study, PEGylated phospholipid formulation containing chemotherapeutic agent paclitaxel was made using organic solution advanced spray drying in a closed mode. This protocol produced micro/nano particles with higher encapsulation efficiency (43-99%). Organic solution advanced spray drying methods have been successfully used by researchers to make nanoparticles of antibiotics and other drugs with and without using phospholipids for pulmonary delivery. Tomodo et al studied the effects of spray drying temperature on particle property and found that PLGA nanoparticles prepared with lactose had best FPF at 90 °C, while those prepared with trehalose had best FPF at 80 °C. Hence, all the variables involved in spray drying process must be carefully considered while designing the study.

Spray freeze drying is a modified spray drying pharmaceutical processing method that can be used to make nanoparticles as well as microparticles under certain conditions. In spray freeze drying, the drug solution is atomized directly into a cryogenic liquid such as liquid nitrogen and frozen into particles. The frozen particles are further subjected to lyophilization to obtain dry powder. Cheow et al prepared spray freeze dried poly (caprolactone) (PCL) nanoparticles containing levofloxacin. They modified a Buchi B-290 spray dryer to freeze dry the formulation, by replacing the drying chamber with a collecting vessel containing liquid nitrogen (i.e. cryogenic liquid). They atomized the liquid slurry through a 1.5 mm nozzle (two fluid atomizer) at a feed rate of 0.24 L/h and atomizing air flow rate of 240 L/h while stirring the liquid nitrogen at 500 RPM. Liquid nitrogen with sample was frozen for 16 hours followed by freeze drying at −52 °C and 0.05 mbar vacuum. This method produced inhalable particles with low density, smooth surface morphology, and good aequous re-dispersibility. The group also found that poly (vinyl alcohol) and mannitol as suitable adjuvants that can be processed with spray freeze drying. Spray freeze drying is not as commonly used as spray drying due to complexity and cost involved. For comparison between spray drying and spray freeze drying pharmaceutical processing methods, the readers are referred to a study conducted by Wang et al for preparation of lipid–polymer hybrid nanoparticles and another study conducted by Maa et al comparing the two methods for the production of inhalable protein powders.

Milling is a commonly used pharmaceutical processing method for primary particle size reduction for generating microparticles (e.g. gas jet milling) and nanoparticles (e.g. nanojet milling). Two types of milling are dry and wet milling. In a study conducted by Onoue et al tranilast, an anti-allergic agent, was prepared into a nanocrystal solid dispersion. There is a concern about this drug’s systemic side effects, which makes inhalation a good choice. Nanocrystal solid dispersion was prepared by wet milling process and dried by freeze drying. The resulting solid-state particles were micronized by gas-jet milling to render them into the respirable size range and blended with large non-respirable lactose monohydrate 50 μm carrier particles. These particles produced dry powder inhalation aerosols with 97.9% emitted dose and 59.4% FPF values. The in vivo characteristics showed a notable performance in anti-inflammatory activity of the formulation. The authors suggested that DPIs of nanocrystal solid dispersions of tranilast could be a simple and safe way to administer the drug with local action and reduced systemic toxicity.

El-Gendy et al formulated budesonide NanoClusters by the wet milling process, while studying the impact of process parameters. Ten hours of milling budesonide reduced the primary particle size from 3 μm to 200-300 nm and had a mean aerodynamic diameter of 1.23 μm (at a concentration of 1.66% w/w). Surface area of the NanoClusters was enhanced with increasing milling time up to 6 hours. NanoClusters had a fine particle fraction of 68-87% and emitted dose higher than 70% for all milling times. Milling of budesonide didn’t affect the physical stability of the drug and it also increased the surface area. This study suggests that wet milling is a suitable method to produce powders for inhalation.

Wang et al prepared polymeric nanoparticle containing paclitaxel of size ~130 nm with 80% encapsulation efficiency by inverse-phase nanoprecipitation method. In this method, an aqueous phase is slowly added to an organic phase and the nanoparticles are allowed to form. Condensation aerosol growth is another approach to make inhalable aerosols. The particles prepared by this method can be inhaled through mouth (i.e. oral inhalation) or nose (i.e. nasal inhalation). There are two approaches to obtain respirable particles where one method consists of the drug alone (enhanced condensation growth) and the other method consists of the drug with excipient (excipient enhanced growth). In enhanced condensation growth (ECG), a submicron size particle aerosol is inhaled with a saturated or supersaturated stream of air that is above body temperature. The inhaled air is cooled in the respiratory track that leads to condensation into the droplets, growth, and enhanced lung deposition. The other hand, excipient enhanced growth (EEG) occurs on aerosol particles that contain a hygroscopic excipient which then absorbs humidity from the lungs leading to aerosol particle growth.

There are two inhalers on the market based on thermal condensation principles, the Staccato® system (Alexza pharmaceuticals) and Aria® system (Chrysalis technology), which use thermal vaporization for aerosol generation. The effect of temperature, initial film thickness and energy on the condensation aerosol was previously studied. The method was successfully used to make ibuprofen and indomethacin nanoparticles for inhalation.

A recent development in nanoparticle fabrication is the top down method of particle replication in non-wetting template shortly called PRINT technology. This technology is used to make monodisperse particles in the solid-state with greater size and shape control. In this method, a master template is made with silicon. Then a suitable polymer is poured into the template and cured to make a mold containing nanocavities. Nanoparticles from here are made by a lamination technique. Solution containing the drug or protein is filled in the cavity which is covered with another layer of empty mold. The two layers of
molds with the drug solution are passed through a roller followed by lyophilization to get solid particles. Studies were conducted to use this technology in making respirable particles for dry powder inhalation.

Other methods used to produce inhalable powders are high-pressure homogenization and supercritical fluid (SCF) technologies. High-pressure homogenization has been used to produce solid-lipid nanoparticles where the drug is dissolved in lipid and homogenized followed by solidification. Recent advancements in supercritical fluid (SCF) extraction have attracted attention for development of nanoparticulate formulations. There are several variations to SCF technology but they all function using the principle of precipitation of the drug when the solvent is extracted with supercritical fluid CO₂.

Future perspective

Inhalable nanoparticles in the solid-state as inhalable powders for targeted pulmonary delivery offer unique advantages and are a new area of research. However, there are limitations. Toxicity of nanoparticles (i.e. nanotoxicity), polymers and other excipients is critical for the development of safe inhalable dry powder inhalation formulations. Nasal delivery of inhalable nanoparticulate powders is gaining research attention recently, particularly in vaccine applications, systemic drug delivery in the treatment of pain, and non-invasive brain targeting. Surface modification and formulation optimization can improve nanoparticle stability, dispersion, and deep lung deposition. Various pharmaceutical processing methods can be employed including advanced spray drying, spray freeze drying, milling, supercritical fluid extraction, condensation aerosol growth, thermal condensation and PRINT technology.

References


