Pulmonary delivery of inhalable nanoparticles: dry-powder inhalers

Pulmonary administration of inhalable nanoparticles (NPs) is an emerging area of interest. Dry-powder inhalers may offer particular advantages for pulmonary administration of NPs. This article reviews research performed on the formulation of inhalable NPs as dry powder to achieve deep-lung deposition and enhance NP redispersibility. Moreover, the article summarizes up-to-date *in vivo* applications of inhalable NPs as dry-powder inhalers.

MHD Kamal Al-Hallak[1,2], Muhammad Khan Sarfraz[1,3], Shirzad Azarmi[1,4], Wilson H Roa[5], Warren H Finlay[6] & Raimar Löbenberg[†1]

[1]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB T6G 2N8, Canada
[2]Faculty of Pharmacy, Damascus University, Damascus, Syrian Arab Republic
[3]Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[4]Research Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[5]Department of Oncology, Cross Cancer Institute, University of Alberta, 11560 University Avenue, Edmonton, AB T6G 1Z2, Canada
[6]Department of Mechanical Engineering, University of Alberta, Edmonton, AB T6G 2G8, Canada
[†]Author for correspondence: E-mail: loebenberg@pharmacy.ualberta.ca

The potential use of nanoparticles (NPs) as a colloidal delivery system for pharmaceutical applications has received considerable interest in the past three decades [1]. In part, this interest emerged due to the ability of NPs to circumvent undesired physiocochemical properties of the active ingredient (AI; e.g., poor water solubility) [2], to protect the AI from early degradation (e.g., enzymatic proteolysis) [3] and to enhance cellular uptake [4]. However, the use of NPs in cancer treatment is still by far the most promising application [5]. This is supported by the ability of NPs to accumulate in cancerous tissues passively by an 'enhanced permeability and retention' (EPR) effect [6] and/or actively by using different targeting probes [7].

Multiple routes of administration can be used for the delivery of NPs, including intravenously, transdermal, ocular, nasal and pulmonary [8–10]. The route of administration chosen should be appropriate to the disease or disorder being targeted and the intended results. Pulmonary delivery is becoming an important route of drug administration for the treatment of intra- and extra pulmonary diseases. This is supported by the lungs' unique characteristics such as large surface area, thin epithelial layer, high vascularization and avoidance of first-pass metabolism [11].

Three approaches to inhaled drug delivery are: nebulizers (solutions inhalers), pressurized metered-dose inhalers (pMDIs) and dry-powder inhalers (DPIs); each category has unique strengths and weaknesses [12]. Nebulizers have been used to deliver inhalable NPs of different types [13–16], but they require bulky compressors or a source of compressed air, so are mainly restricted to hospitals and ambulatory care settings; children, the elderly and people with inadequate physical or mental ability to use MDIs and DPIs may use nebulizers.

Pressurized metered-dose inhalers are the most popular inhaled drug-delivery system. However, it is challenging to formulate NPs for pMDIs as they are typically unstable due to the potential for sedimentation, crystal growth and polymorphism. In addition, pMDIs emit dosages at high velocity, which makes deposition in the oropharynx more likely [17,18]. The aforementioned characteristics make pMDIs less suitable for delivery of inhalable NPs.

Dry-powder inhalers were introduced to overcome some of the drawbacks associated with pMDIs [19,20]. DPIs offer higher formulation stability and can provide deep-lung deposition [21,22]. A step forward in the pulmonary delivery of DPIs was the introduction by Edwards *et al.* of large porous particles of small mass density and large geometric diameter. Due to their physical characteristics, LLPs were able to escape the lung's natural clearance mechanisms and achieve deep-lung drug release [23].

The delivery of NPs from DPIs combines the advantages of nano-scale formulation and the localized effect of NPs in the lungs, especially in the treatment of tuberculosis (TB) [24], cystic fibrosis [25,26] and lung cancer [27]. The pulmonary delivery of NPs showed clear advantages over the use of microparticles and other inhalable formulations, such as enhanced activity [28], increased cellular uptake [29–31], elevated immunological response [32,33], longer lung retention [34], modified pharmacokinetics, an extended release profile [25,35,36], improved tolerability and reduced toxicity [27].

However, the direct inhalation of NPs from DPIs is not feasible. Due to their submicron diameter, they are mostly exhaled after inhalation, with minimal deep-lung deposition [37]. Other studies showed that NPs tend to strongly agglomerate when formed as dry powder;

FUTURE SCIENCE part of fsg

Key Terms

Redispersibility: Ability of nanoaggregates, nanocomposites, or microcarrier particles to release nanoparticles similar in characteristics to the original nanoparticles upon exposure to humidity *in vitro* or to lung fluids *in vivo*.

Hollow nanoaggregates: Aggregations of nanoparticles in the form of hollow particles in which the shells are made of layers of nanoparticle aggregations.

Nanocomposites: Nanoparticles agglomerated with other excipients to bind nanoparticles together to form porous particles with low density for deep-lung deposition.

moreover, the dry powder is difficult to handle and has low ability to release NPs similar in characteristics to the original NPs (low **redispersibility**) [38–41]. The phagocytic clearance of inhaled NPs may also hinder the maximum efficacy of NP treatment. On the other hand, targeting macrophages might be preferable in lung diseases such as TB. Some studies showed that NPs can modify cytokine profiles released from macrophages in a way that helps to reject cancer [11].

Different pharmaceutical approaches have been developed to overcome the difficulties of formulating NPs in DPIs for pulmonary delivery. These pharmaceutical interventions mainly sought to increase the mass median aerodynamic diameter (MMAD) of the inhalable powder to between 1–5 µm, optimize the fine particle fraction (FPF) and enhance the redispersibility of NPs after pulmonary delivery. This review discusses several pharmaceutical interventions to achieve deep-lung deposition of inhalable NPs and enhance NP redispersibility after pulmonary delivery.

Enhancing deep-lung deposition

■ Large porous NP aggregates

Large porous NP aggregates are inhalable-powder dosage forms comprised mainly of NPs and designed for pulmonary delivery. NPs in the form of LPNPs are held together by physical means, such as van der Waals forces (as in **hollow nanoaggregates**), or by using binders such as polymers, phospholipids, or sugars (as in **nanocomposites**).

Hollow nano-aggregates & Trojan particles

Tsapis *et al.* first described hollow nanoaggregates as Trojan particles in 2002 [38]. Hollow nanoaggregates are hybrid LPNPs made of hollow NP aggregations that combine the delivery potential of NP systems with the ease of flow of large porous microspheres.

Formulation of hollow nanoaggegates & Trojan particles

Trojan particles are nanoaggregates prepared by spray drying an NP dispersion to form hollow microparticles in which the crust or shell are made of layers of NP aggregations. In general, the spray-drying technique atomizes the NP dispersion mixture and other excipients to a spray form that is put immediately into thermal contact with a hot gas, resulting in the rapid evaporation of the droplets to form dried solid particles. The dried particles are then separated from the gas by means of a cyclone. To obtain hollow nanoaggregates, the drying time of the sprayed droplets should be sufficiently shorter than the characteristic time for redistribution of NPs by diffusion within the drying droplet [38]. Tsapis *et al.* proposed two critical times in the drying process to produce such particles. The first is the time required for a droplet to dry and the second is the time required for the NPs to diffuse from the edge to the center of the droplet. The ratio of these two characteristic times (the first over the second) defines a Péclet number (Pe). NP aggregations in the form of hollow microparticles will be produced when the spray-drying settings result in a large enough Pe (Pe >> 1). Three main variables were controlled in order to maximize the Pe and to form hollow particles: inlet temperature, droplet atomization efficiency and feed rate. High inlet temperature (110°C) could be accompanied by lower droplet atomization efficiency and a higher feed rate (70 ml/min), whereas lower inlet temperature (95°C) required higher atomization efficiency and a lower feed rate (40 ml/min). Kawakami *et al.* proposed a more efficient drying process and, consequently, a shorter droplet-drying time by adding the surfactant sodium dodecyl sulfate (SDS) to the sprayed mixture [42]. Trojan particles produced by Tsapis *et al.* were spherical in shape and had a mass density around unity and an MMAD, approximately 3 µm, which is suitable for deep pulmonary delivery. The shell wall of the Trojan particle was made of several layers of NP aggregations. In spite of the appealing characteristics of Trojan particles produced by Tsapis *et al.*, their ability to release and redisperse NPs *in vivo* remains uncertain. The *in vitro* redispersibility test was performed by vortexing the dry powder of Trojan particles in a mixture of ethanol:water (70:30 by volume). This may be inappropriate as it does not represent the actual redispersion mechanism in lung fluid, where dispersion is caused by spontaneous particle wetting. The presence of a water-soluble excipient (mannitol) that forms 'excipient bridges' interconnecting the NPs was found by Kho *et al.* to be required to enable spontaneous redispersion [43]. Hadinoto *et al.* showed that the morphology and degree of hollowness of nanoaggregates prepared by spray drying depend on the chemical nature of the NPs, but not on their size [44]. Polyacrylate, silica and polystyrene NPs of similar size and concentration produced different hollow particles under

the same spray-drying condition. This can be explained by the differing types of stabilizing forces imposed by the differing chemical natures of the NPs. In addition to the inlet temperature and feed rate, Cheow *et al.* studied the effect of feed concentration, feed pH and the ratio of the gas-atomizing flow rate to the feed rate on the morphology of silica hollow nanoaggregates [45]. This study showed that there is a minimum feed concentration below which Trojan particles cannot be formed (0.18%, w/w for silica NPs). The optimal spray-drying formulation parameters from which hollow spherical nanoaggregates with a narrow bimodal distribution were produced were identified by Cheow *et al.* as: inlet temperature 120°C, pH 9, feed concentration 0.8% (w/w), feed rate $0.18 \times 10^{-3} m^3/h$ and gas atomizing flow rate 0.332 m^3/h. It has been shown that the degree of hollowness (ψ), which is defined as the ratio of the effective density ρ_e to the true density ρ_{true} of the nanoaggregates, can be increased by using phospholipids during the spray-drying process [46]. The degree of hollowness of hollow nanoaggregates made of polyacrylate NPs with size close to 160 nm was increased from 0.1 to 0.4 as the phospholipid concentration increased from 0 to 60% w/w. In another study, Kho *et al.* showed that the redispersibility of the hollow nanoaggregates was related to the degree of hollowness [47]. In this study, the degree of hollowness was controlled by the NP:mannitol ratio. Two parameters were proposed by Kho *et al.* to evaluate the redispersibility: (1) the ratio of NP diameter retrieved from the nanoaggregates (S_f) to original NP diameter (S_i), where a S_f:S*i* ratio ≈ 1 suggests that the nanoaggregates are fully redispersible into the primary NPs; and (2) the turbidity level of an NP dispersion produced by hollow nanoaggregates upon exposure to moisture, with higher turbidity levels indicating bigger NPs and poor dispersibility. Nanoaggregates with a higher shell thickness:particle radius ratio exhibited weaker redispersibility due to poor particle wetting. Hollow nanoaggregates with the highest redispersibility were obtained from spray drying at 0.72% (w/w) NP concentration and a silica:mannitol ratio of 1:4. The thickness of the hollow nanoaggregate shell was found to be influenced more by process parameters acting at the colloidal level (e.g., pH) than by the spray-drying operation settings [48]. The effect of different excipients on the morphology and redispersibility of hollow nanoaggregates of silica NPs was assessed by the same group in a different study [49]. The study compared leucine, mannitol and lactose, and a mixture of these materials, and determined the NP:excipient concentration ratio required to produce hollow nanoaggregates with the best morphology and redispersibility characteristics. Mannitol alone produced particles with large, hollow and spherical morphologies, but with poor redispersibility. Lactose alone showed highly redispersible particles at the expense of morphology. The best results were achieved with a multiple-excipient formulation of leucine and lactose at a 1:6 concentration ratio. The hydrophobic leucine was included in the formulation to serve as a water repellent to reduce the moisture uptake of the highly hygroscopic spray-dried lactose, to reduce particle cohesiveness and to improve particle morphology.

In vivo *studies*

The only *in vivo* study of hollow nanoaggregates after pulmonary delivery was performed by Sung *et al.* [34]. This study investigated the pharmacokinetics of rifampicin, loaded in polylactic-co-glycolic acid (PLGA) Trojan particles in guinea pigs after pulmonary delivery. The results showed a prolonged rifampicin presence in the lungs with detectable drug levels up to 8 h after pulmonary delivery. The pulmonary delivery of the free rifampicin in porous particles resulted in much lower drug levels in lung tissues, providing evidence for the advantage of using inhalable NPs over microparticles. After 8 h, hollow nanoaggregates containing 80% NPs by weight were associated with higher rifampicin concentrations in lung tissues than nanoaggregates containing 40% NPs, due to delayed release of rifampicin from the NPs. Plasma concentrations of rifampicin after pulmonary administration of hollow nanoaggregates (40 and 80%) to guinea pigs were higher at all times than after the standard oral administration of equivalent doses (2.5 mg/kg) of rifampicin suspension.

Nanocomposites

Nanocomposites are made by binding NP agglomerations with other excipients to form particles appropriate for pulmonary delivery and deep-lung deposition. The main difference between nanocomposites and Trojan particles is that nanocomposites are not hollow particles, yet they possess the required aerodynamic characteristics for pulmonary delivery due to their porous texture. Nanocomposites can be made out of polymeric NPs or NPs of the AI.

Formulation of nanocomposites

Spray drying

Tomoda *et al.* studied the effect of inlet temperature and the weight ratio of NP to excipients on nanocomposites made of PLGA NPs with a sugar (lactose or trehalose) binder [50]. The results showed that the optimal inlet temperature depends mainly on the size range of the primary NPs. Bigger (400 nm) PLGA NPs showed a higher ability to tolerate elevated inlet temperatures, whereas smaller (200 nm) NPs formed nanocomposites that were not redispersible when the inlet temperature was 80°C and above. For the 400 nm NPs, the best nanocomposites were obtained with trehalose, an inlet temperature of 90°C and a 45% NP:excipient weight ratio. For the 200 nm NPs, the best results were obtained with trehalose and a 70°C inlet temperature, regardless of the NP:excipient weight ratio. Another study found that lower inlet temperatures yielded PLGA nanocomposites with larger sizes but better redispersibility, and that the optimal inlet temperature was dependent not only on the size of NPs, but also on the type of binder used in the nanocomposites (80°C for trehalose and 90°C for lactose) [51].

The maximum tolerable inlet temperature during spray-drying processes has been shown to be related to the glass transition temperature (T_g) of the polymer [52]. In this regard, Yamamoto *et al.* showed that PLGA with a molecular weight (MW) of 20,000 or lower exhibited minimum heat tolerance, as the nanocomposites obtained at inlet temperatures higher than 45°C showed no ability to redisperse to release original NPs. However, NPs made of a higher molecular weight polymer (120,000) were able to form redispersible nanocomposites at a higher inlet temperature (70°C). The composition of the AIs may also require the use of low inlet temperatures. Jensen *et al.* studied the spray drying of siRNA-containing PLGA NPs using a very low inlet temperature (45°C) in order to avoid the decomposition of siRNA. This study showed that when using a low inlet temperature, the best nanocomposites were obtained using a high concentration of mannitol (30 mg/ml) and a low NP:excipient ratio (20%) [53].

Muttil *et al.* reported nanocomposites of PLGA NPs containing CRM-197, a diphtheria antigen [33] and PLGA–polyethylene glycol (PEG) NPs containing recombinant hepatitis B surface antigen [32] used as inhaled vaccinations. Nanocomposites prepared by Muttil *et al.* were obtained by spray drying a mixture of NPs dispersed with an L-leucine solution; the ratio of NPs to L-leucine was kept at 25:75 for PLGA NPs and at 10:90 for PLGA/PEG NPs. Other experimental settings of the spray-drying procedure were: inlet temperature 95°C and 80°C, feed rate 30 ml/min and air flow rates of 100 and 98 kg/h for PLGA and PLGA–PEG NPs, respectively. Both nanocomposite powders (PLGA and PLGA–PEG) showed almost the same characteristics: MMAD was approximately 5 µm and the $FPF_{<5.8\mu m}$ was approximately 50%. No redispersibility data were reported in either study.

To avoid rapid alveolar macrophage uptake and clearance upon pulmonary delivery, the nanocomposites should disintegrate rapidly and release the NPs at the site of action. Disaggregation is dependent on the solubility of the binder used during the spray-drying process. Therefore, Lebhardt *et al.* investigated the effects of using different excipients with different water solubilities on the redispersibility of nanocomposites [54]. Compared to lactose and mannitol, α-cyclodextrin produced PLGA nanocomposites were readily redispersed within 5 min by gently shaking; PLGA NPs of approximately 200 nm were released.

Spray-freeze drying

During the spray-drying process, NPs are exposed to harsh physical conditions such as thermal stress during droplet drying, high shear stress in the sprayer nozzle and high adsorption at the greatly expanded liquid–air interface of the spray solution [40]. These conditions might affect both the polymer matrix and the AIs of the NPs and the redispersibility of the nanocomposites. Instead of solvent evaporation (as in spray drying), atomized droplets created by spraying are instantly frozen in liquid nitrogen. The particles are lyophilized to remove the solvent and create porous spherical particles suitable for inhalation. Alternatively, a cold gas can be used to both freeze and dry the particles [55].

Cheow *et al.* reported a spray-freeze drying method to prepare nanocomposites of thermally sensitive polycaprolactone (PCL) NPs, as the low melting point of PCL limits the use of spray drying due to the high temperature required [56]. The results showed that nanocomposites produced by spray-freeze drying exhibited higher aqueous redispersibility than nanocomposites of the same formulation obtained by spray drying. Two different excipients were used to stabilize the nanocomposites during the spray-freeze drying process; mannitol and polyvinyl alcohol

(PVA), with variable NP: excipient ratios (4:1, 3:2 and 3:7). Increasing the mannitol concentration in the feeding solution from 0 to 70% enhanced the redispersibility from 30% to 85%. The best redispersible nanocomposites were obtained with high PVA concentrations (60% to 70%) in the feed solution.

Controlled flocculation & milling

Controlled flocculation has been used to prepare nanocomposites of different AI: budesonide [57], ciprofloxacin [58], paclitaxel [59], diatrizoic acid [60] and nifidipine [61]. NPs were prepared by antisolvent precipitation (budesonide, diatrizoic acid, paclitaxel and nifidipine) or sonication of colloidal solution (ciprofloxacin). The main flocculating agents used to induce NP agglomeration were L-leucine solution (1%, w/v) and solid sodium chloride crystals. The amount of L-leucine added was adjusted to an AI:leucine ratio of 1:1. The L-leucine solution was added slowly to the NP suspension during homogenization and then the flocculated NP suspension was lyophilized to a dry powder. The MMAD for budesonide nanocomposites prepared under the aforementioned experimental settings was 2.1 ± 1.8 µm, which is suitable for deep -lung deposition [57]. The dissolution rates of nanocomposites in all the studies were faster than unprocessed drug and slower than pure NPs.

Aillon *et al.* reported a wet-milling procedure to prepare nanoclusters of N1177 (a diatrizoic acid derivative), to be used by pulmonary delivery as a contrast agent for thoracic computed tomography (CT) [62]. Drug suspension samples were collected at different intervals of the milling procedure. The results showed that particles decreased in size within the first 30 min, followed by an increase in size over the next 2 h. During this process, discrete NPs were not observed in the suspension, rather, they appeared to be agglomerated into low density clusters. No excipients other than the grinding media were used during this procedure. The milling procedure was followed by lyophilization to convert the nanocluster suspension into a dry powder for inhalation. The reported MMAD of the final nanoclusters was 4.2 ± 0.1 µm and the $FPF_{<5.7\mu m}$ was 75 ± 1, characteristics suitable for deep-lung deposition.

In vivo *studies of nanocomposites*

The distribution of insulin-loaded PLGA nanocomposites in the lungs after intratracheal administration in rats was studied by Yamamoto *et al.* The results of the study showed that the percentage of nanocomposites deposited in bronchioles and alveoli was approximately 80%. These nanocomposites showed extended glycemic control after pulmonary delivery (up to 12 h) compared with insulin solution after intratracheal (up to 6 h) and intravenous (iv.; up to 4 h) administration [52].

Tomoda *et al.* studied the body distribution of TAS-103 (an anticancer agent) after pulmonary delivery in the form of nanocomposites [63]. This study showed that the TAS-103 concentration in the lungs after pulmonary delivery was 13 times higher than after iv. injection of the same dose. The efficacy (as a lung cancer treatment) of TAS-103 loaded in inhalable nanocomposites was not reported.

Muttil *et al.* evaluated the immune response for nanocomposites of PLGA NPs containing diphtheria antigen [33] and PLGA–PEG NPs containing recombinant hepatitis B surface antigen after pulmonary delivery [32]. Relative IgG in the blood at different time points and IgA titers in the bronchio–alveolar lavage fluid at the end of the study were evaluated and compared with an intramuscular (im.) administration. Both studies reported an increase in IgA in the lungs but lower serum IgG levels when compared with im. vaccination. Even though the IgG level induced by inhalable nanocomposites was lower than the humoral response induced by im. administration, the IgG level induced by pulmonary vaccination was sufficient to provide protection in both cases. The authors claimed that higher mucosal IgA levels provide higher protection from infectious agents entering through the mucosal route.

The efficacy of using N1177 nanoclusters as a contrast agent for thoracic CT in rats was evaluated by Aillon *et al.* [64]. Analysis of the CT images revealed a 118-Hounsfield units (HU) difference between the images taken prior to dosing (-620 HU) and 1 h postinhalation (-502 HU). This excellent contrast enhancement was well above the 30 HU minimum needed for contrast. A histology study showed no acute lung toxicity in tissue collected from rats 2 h postinhalation.

Mizoe *et al.* studied the pharmacokinetics of pranlukast nanocomposites after intratracheal administration in rats [65]. Pranlukast is a leukotriene antagonist used for the treatment of bronchial asthma with low bioavailability after oral administration. The results showed that the area under the curve (AUC) per dose following pulmonary administration of the

Key Term

Microparticle carriers: Particles made of one or a mixture of excipients that work as vehicles to enhance deep-lung deposition of nanoparticles.

nanocomposites was approximately 100-fold higher than the AUC per dose for oral administration of pranlukast powder at a dose of 2.5 mg/kg. The extended absorption pattern (6 h after pulmonary delivery) suggested that pranlukast NPs were retained on the pulmonary mucous and slowly dissolved, resulting in prolonged absorption.

■ Microparticle carriers

This pulmonary delivery system aims to overcome difficulties presented by the unsuitable diameter of NPs for pulmonary delivery. In this approach, deep-lung deposition is achieved by loading NPs in **microparticle carriers** that possess optimal dispersability and deposition properties.

Formulation of NP-loaded microparticles

Spray drying

*Sham et al. re*ported a spray-drying technique of loading different types of NPs, gelatin and poly(isobutylcyanoacrylate) (PIBCA) into lactose microparticles [66]. The MMAD of the resultant microparticles was 3.0 ± 0.2 µm and the $FPF_{<5.6\mu m}$ was 40%, characteristics suitable for deep-lung deposition. An NP size increase of approximately 30% was noticed after the spray-drying process. The size of the NPs in the droplet, droplet size, droplet viscosity, drying temperature, gas flow rate and the addition of surfactant all crucially affect the morphology of microparticle carriers produced by spray drying [67]. Grenha *et al.* investigated the effects of distinct formulation variables (aerosol excipient/NP theoretical ratio, concentration of the spray-drying suspension) on the microspheres' aerodynamic and morphologic properties [68]. This study showed that the incorporation of NPs into a solid structure improved the mannitol microsphere morphology. Microspheres showed more defined limits and spherical shape as the mannitol–NP ratio decreased from 100/0 to 80/20. Any increase in the NP concentration beyond this ratio did not improve the morphology. In terms of the structure of the microparticles, it was shown that mannitol is distributed throughout the microsphere and NPs are homogeneously mixed with mannitol. Moreover, both components were present on the microsphere surface, with mannitol being present to a higher extent than NPs [69]. Takashima *et al.* investigated the use of cationic PLGA NPs instead of plain PLGA NPs for gene delivery by pulmonary administration [28]. The cationic polymer, polyethyleneimine (PEI), was mixed with PLGA during NP preparation to enable (plasmid DNA) pDNA (which is negatively charged) encapsulation. PLGA–PEI NPs loaded with pDNA were spray dried with mannitol to produce microparticles. The spray dryer was operated with a minimum inlet temperature (60°C) and a minimum feed rate (5 ml/min) to protect the pDNA from degradation during the spray-drying process. An *in vivo* gene expression study showed that pDNA remained intact during the spray-drying process.

In order to design an extended-release pulmonary delivery system, Li *et al.* investigated the effect of adding chitosan to mannitol to form microparticles loaded with honokiol NPs [70]. *In vitro* release of honokiol from mannitol–chitosan microparticles showed longer delayed release as the chitosan content of microparticles increased from 20 to 30%.

The preparation of simultaneously manufactured nano-in-micro (SIMANIM) particles was reported by Kaye *et al.* [71]. As the name implies, NPs and microparticle carriers are formed simultaneously. This is achieved by spray drying a double emulsion (water–oil–water) of the AI (IgG), the polymer solution (PLGA) and the solution of the bulking material (lactose). Using this method, Kaye *et al.* were able to use a high inlet temperature (100°C) with no evidence of IgG degradation. Ohashi *et al.* compared using the traditional two-fluid nozzle and the new four-fluid nozzle to prepare PLGA NP-containing mannitol microparticles [72]. The four-fluid nozzle has two fluid and two gas feed lines. This nozzle design allows the use of different solvents during the spray-drying process and reduces the time of contact between incompatible materials. No significant differences were observed between microparticles prepared by the two-fluid or four-fluid nozzle method.

El-sherbiny *et al.* reported a novel approach to obtain respirable/swellable microspheres for controlled-release pulmonary drug delivery [73]. These microparticle carriers have respirable aerodynamic sizes when dry but large geometric sizes when swollen after deposition in the moist lung. A large geometric diameter enables particles to evade macrophage uptake during delivery. Briefly, the chemically modified chitosan NPs were loaded in a sodium alginate microparticle carrier by spray drying. The obtained dry powder was soaked in 10 ml of 0.1 M $CaCl_2$ solution as an ionotropical crosslinker and the mixture was freeze dried to obtain inhalable dry powder. Upon exposure to humidity, dry alginate

microparticles showed an increase in diameter from approximately 4 to 84 µm. Release of the encapsulated drug was shown to depend mainly on enzymatic degradation.

Spray freeze-drying

This technique is a different approach to prepare microparticle carriers using a mixture of NPs and a bulking material. Azarmi *et al.* reported a method to prepare PIBCA NPs loaded in effervescent microparticle carriers; these carriers are discussed later in more detail [74]. Spray-freeze drying was employed with a four-fluid nozzle [75].

The main advantages of spray-freeze drying over spray drying are prevention of NP aggregation at an evaporation front during particle formation and avoidance of elevated temperatures which improves the stability of both the polymer and AI [76]. Similar to spray drying, the freeze-drying process could destabilize NPs. During the freezing of a sample, there is a phase separation into ice and cryoconcentrate that contains NPs, which promotes NP aggregation [77]. Therefore, use of a cryoprotectant such as dextran is usual during lyophilization to avoid NP aggregation. Different parameters of the spray-drying process (e.g., type of polymer and pH of the aqueous dispersion) and their effects on the quality of the NP dry powder were previously studied and reviewed [78].

Using oils as extractable porogens

Arnold *et al.* reported a unique method of creating porous microparticle carriers using porogenic oils [79]. In this method, PLGA microparticles were prepared by creating a mixture of PLGA solution and ciprofloxacin NP suspension. Porogenic oil (canola or silicone) was then added and the mixture was sprayed using a small-gauge needle or circular orifice while an ultrasonic transducer controlled by a frequency generator disrupted the stream into uniform droplets. The oil was then extracted with heptane to produce large porous particles loaded with cipro NPs. While canola oil led to particles formed with a porous, web-like internal structure, hollow particles resulted from the use of silicone oil. In general, this method produced large (~10–15 µm), low-density (1.11 to 0.95 g/cm^3) microparticle carriers of PLGA, depending on the oil used. The main drawback of this method was the low (maximum approximately 9%) loading efficiency of cipro NPs into the PLGA microparticle carrier.

Enhancing NP redispersibility & effervescent technology

The purpose of the pharmaceutical formulations discussed above is to carry NPs and deposit them deep in the lung. Thereafter, NPs are supposed to be released and readily redispersed without a significant increase in size. Spray drying or spray-freeze drying results in variable amounts of NP aggregation and, thus, a delay in the redispersibility process and an increase in NP size. Therefore, excipients such as a fast dissolving matrix (spray-dried lactose and mannitol and cyclodextrins) [40,54], water-soluble polymers (polyvinyl alcohol and PEG 6000) [80] or different surfactants (pulmonary surfactant components and polysorbate-80) were used to enhance redispersibility [43]. Some excipients (e.g., polysorbate-80) that demonstrated *in vitro* efficacy might be associated with *in vivo* toxicity [81].

An approach developed by Ely *et al.* [80] depends on adding an effervescent pair in the formulation during the spray-drying process. Upon spray drying, microcarrier particles that contain NPs will possess effervescent properties that allow them to actively release NPs once they are in contact with any source of water or humidity, such as the physiological fluids in the lungs. The effervescent components used were citric acid and anhydrous sodium carbonate; citric acid was added to a poly(isobutylcyanoacrylate) (PIBCA) NP dispersion to form mixture (A) and ammonium hydroxide (ammonia) solution with anhydrous sodium carbonate was added to a solution of spray-dried lactose monohydrate to form mixture (B). Mixtures (A) and (B) were mixed immediately before the spray-drying process. The role of ammonia solution was to inhibit the effervescent reaction prior to the spray-drying process; it was removed during the spray-drying process as it has a low boiling point (~38°C). Scanning electron microscope and confocal microscopy images showed microcarrier particles with diameters under 5 µm, spherical in shape, with NPs distributed continuously throughout the matrix. The average MMAD for effervescent carrier particles smaller than 5.6 µm was 2.17 ± 0.42 µm; the $FPF_{<5.6\mu m}$ was approximately 46.47 ± 15% and the geometric standard deviation (GSD) was 2.00 ± 0.06. Upon exposure to humidity, the dry powder actively released NPs through gas bubbles with an average diameter of 30 µm, produced by the effervescent reaction as shown in **Figure 1**. The

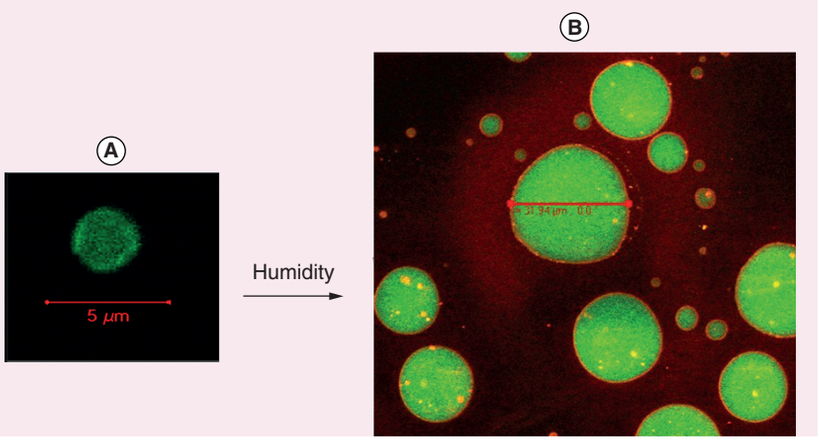

Figure 1. Confocal microscopy of (A) an efferevescent carrier particle with nanoparticles continuously distributed through out the carrier particle and (B) effevescent particles exposed to humidity showing nanoparticle distributed throughout the gas buubles with average diameter of 30 µm.
Modified with permission from [80]. © Elsevier Publishers.

size of the NPs was not affected by the spray-drying technique, as NPs released from the effervescent dry powder were similar in size to NPs observed before spray-freeze drying.

In order to avoid the disadvantages of spray drying, Azarmi *et al.* used the same technique but replaced spray drying with spray-freeze drying and tested the tolerability of the effervescent powder *in vivo* using animal models [74]. For this purpose, blank NPs were loaded in effervescent microparticles of lactose monohydrate using spray-freeze drying as shown in **Figure 2**. The microcarrier particles showed optimum MMAD (4.80 ± 2.12 µm) and redispersibility properties (157 ± 12 and 162 ± 16 before and after spray-freeze drying, respectively).

■ *In vivo* studies

Li *et al.* studied the pharmacokinetics and pharmacodynamics of thymopentin-loaded solid–lipid NPs (SLN) in the form of inhalable microparticles in rats [82]. The inhalable SLN microparticles exhibited slower absorption, in which the serum concentration increased gradually to 52 ng/ml over 2 h followed by sustained drug release. Moreover, the C_{max}, AUC and mean resident time significantly increased after the administration of inhalable SLN microparticles compared with a group that was administered intravenously. Along with improved pharmacokinetics, inhalable SLN microparticles possessed enhanced thymopentin efficacy as an immunomodulator. Thymopentin delivered in the form of inhalable SLN microparticles showed significantly higher effects on modulating superoxide dismutase activity to normal and normalizing the $CD4^+/CD8^+$ in immunodepressed rats compared with the iv. group.

In a mouse model, Takashima *et al.* performed an *in vivo* transfection study to compare the efficacy of PLGA/PEI NPs loaded in mannitol microparticle carriers and PLGA/PEI microparticles as luciferase pDNA carriers [28]. The results showed that mice treated with NPs loaded in a microparticle carrier had significantly higher lung luciferase activity than mice treated with

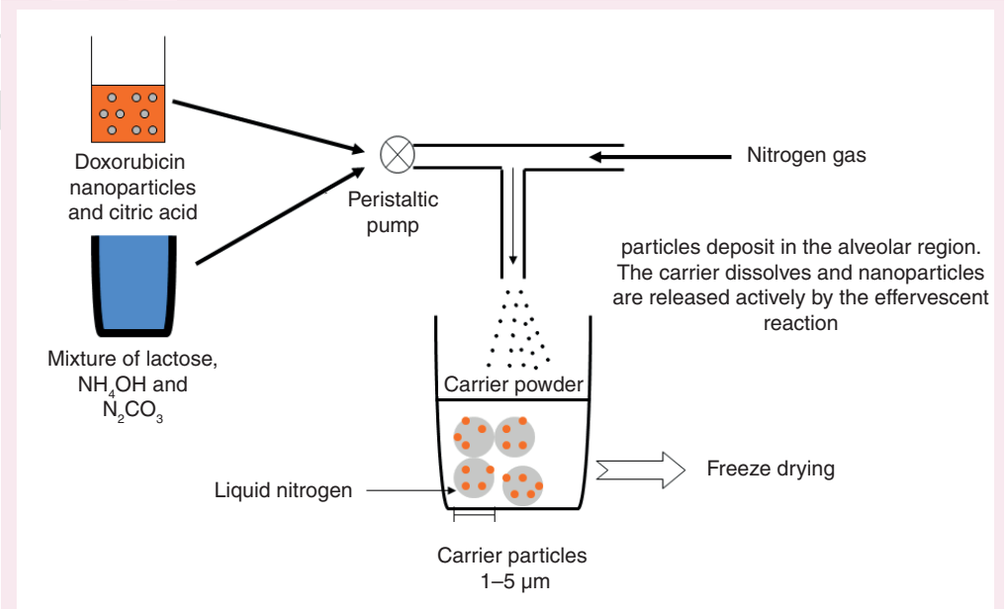

Figure 2. Spray-freeze drying process for effervescent inhlalable nanoparticle powder.

microparticles loaded with free pDNA, indicating that particle size affects gene expression efficiency *in vivo*.

Azarmi *et al.* evaluated the tolerability of inhalable NPs loaded in effervescent microparticles in a mouse model [74]. The treated mice were observed for body weight and changes in morbidity scores. The results showed that effervescent carrier powder was very well tolerated as no significant changes in the morbidity scores were observed over a four-week period. The potential use of effervescent inhalable NPs to treat lung cancer was also assessed *in vivo* by Roa *et al.* [27]. Balb/C nude mice were injected with non-small cell lung cancer. One week after cancer cell inoculation, the mice were treated with 1 mg of doxorubicin (DOX)-loaded NPs carried in effervescent and noneffervescent carrier microparticles made of lactose (12). The efficacy of the two formulations of inhalable NPs was compared with different conventional treatments (iv. injection of DOX solution and DOX NPs). Inhalable NPs, in general, showed an increase in efficacy and a decrease in toxicity compared with inhalable DOX dry powder and conventional tail-vein injections of DOX solution. Animals treated with effervescent inhalable NPs showed a higher survival time than ones receiving the same dose of the noneffervescent formulation (mean time of survival of 116.3 and 83.6 days, respectively) as shown in **Figure 3.** This could be explained by the ability of the effervescent microparticle carriers to release NPs actively which results in higher lung distribution and avoidance of macrophage uptake. Inhalable NPs in the form of effervescent carrier powder showed significantly lower cardiac toxicity in comparison with same dose of injected or inhaled DOX.

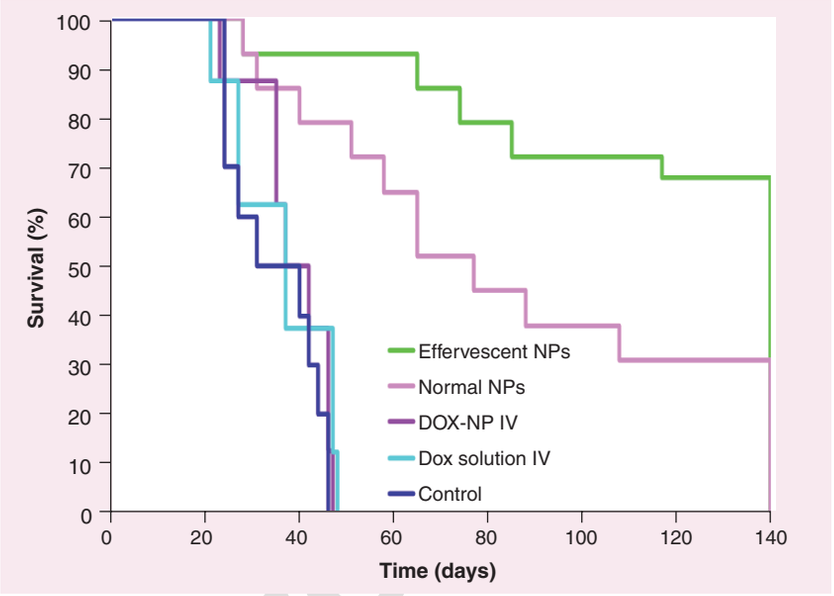

Figure 3. Percent animal survival versus time. Animals were treated either with effervescent inhalable doxorubicin nanoparticle powder (Inh Eff NPs), inhalable doxorubicin nanoparticle powder (Inh NPs), doxorubicin-loaded nanoparticles iv. (Dox NP iv.) and doxorubicin solution (Dox Sol IV) or non-treated control group (no treatment) (12).
Modified with permission from [27] © Elsevier Publishers.

Conclusion & future perspective

The local pulmonary delivery of NPs to the lungs has potential for use in multiple lung disorders and diseases. The local pulmonary delivery of dry-powder formulations of NPs to the lungs has potential for use in multiple lung disorders and diseases. Different pharmaceutical approaches were tested to convert NPs to a dry powder suitable for deep-lung deposition. It has been shown that different experimental settings affect NP–NP and NP–excipient interactions to form particles of different characteristics. Similarly, different approaches were implemented to enhanced particle redispersability; among them, effervescent inhalable NP powder is currently the only active release mechanism for NPs. The enhanced NP redispersibility provided in effervescent technology helps overcome difficulties resulting from NP aggregation during spray drying and spray-freeze drying. Inhalable NPs in the form of dry powder has been tested *in vivo* using different animal models. The results of these studies indicate that inhalation is a noninvasive approach for pulmonary drug delivery. Promising future applications include evaluating the pulmonary delivery of anticancer agents (in the form of DPIs) for lung cancer treatment and treatment of lung disorders that require cell targeting and localized mechanisms of action in humans.

Acknowledgements

MHD Kamal Al-Hallak acknowledges the receipt of a Damascus University scholarship.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- This article reviews pharmaceutical interventions utilized to formulate nanoparticles (NPs) in dry powder for pulmonary delivery.
- The pharmaceutical interventions were divided into two categories:
  - Enhancing deep-lung deposition.
  - Enhancing NPs redispersibility.
- Dry-powder inhalers were categorized depending on the microstructure of the final powder into hollow nanoaggregates, nanocomposites and microparticle carriers.
- Each category was discussed in detail in regard to formulation aspects (drying technique, pH, feed rate, temperature, nature of nanoparticle, the effect of different excipients).
- In addition to optimizing deep-lung deposition, approaches to enhance NPs redispersibility were discussed and compared with focus on using an effervescent technique.
- Moreover, the article reviews up-to-date *in vivo* results of using inhalable NPs, in the form of dry-powder inhalers, for different therapeutic and diagnostic purposes in animal models.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Davis ME, Chen ZG, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discov.* 7(9), 771–782 (2008).

2 Fukami T, Ishii T, Io T *et al.* Nanoparticle processing in the solid state dramatically increases the cell membrane permeation of a cholesterol-lowering drug, probucol. *Mol. Pharm.* 6(3), 1029–1035 (2009).

3 Khan JA, Kainthan RK, Ganguli M, Kizhakkedathu JN, Singh Y, Maiti S. Water soluble nanoparticles from PEG-based cationic hyperbranched polymer and RNA that protect RNA from enzymatic degradation. *Biomacromolecules* 7(5), 1386–1388 (2006).

4 Wong HL, Bendayan R, Rauth AM, Xue HY, Babakhanian K, Wu XY. A mechanistic study of enhanced doxorubicin uptake and retention in multidrug resistant breast cancer cells using a polymer–lipid hybrid nanoparticle system. *J. Pharmacol. Exp. Ther.* 317(3), 1372–1381 (2006).

5 Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. *Adv. Drug Deliv. Rev.* 56(11), 1649–1659 (2004).

6 Gipps EM, Groscurth P, Kreuter J, Speiser PP. Distribution of polyhexylcyanoacrylate nanoparticles in nude mice over extended times and after repeated injection. *J. Pharm. Sci.* 77(3), 208–209 (1988).

7 Moghimi SM, Hunter AC, Murray JC. Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol. Rev.* 53(2), 283–318 (2001).

8 Calvo P, Vila-Jato JL, Alonso MJ. Comparative *in vitro* evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers. *J. Pharm. Sci.* 85(5), 530–536 (1996).

9 Cosco D, Celia C, Cilurzo F, Trapasso E, Paolino D. Colloidal carriers for the enhanced delivery through the skin. *Expert Opin. Drug Deliv.* 5(7), 737–755 (2008).

10 Azarmi S, Roa WH, Lobenberg R. Targeted delivery of nanoparticles for the treatment of lung diseases. *Adv Drug Deliv Rev* 60(8), 863–875 (2008).

11 Al-Hallak KM, Azarmi S, Anwar-Mohamed A, Roa WH, Lobenberg R. Secondary cytotoxicity mediated by alveolar macrophages: a contribution to the total efficacy of nanoparticles in lung cancer therapy? *Eur. J. Pharm. Biopharm.* 76(1), 112–119 (2010)

12 Chow AH, Tong HH, Chattopadhyay P, Shekunov BY. Particle engineering for pulmonary drug delivery. *Pharm. Res.* 24(3), 411–437 (2007).

13 Dailey LA, Schmehl T, Gessler T *et al.* Nebulization of biodegradable nanoparticles: impact of nebulizer technology and nanoparticle characteristics on aerosol features. *J. Control. Release* 86(1), 131–144 (2003).

14 Shrewsbury SB, Bosco AP, Uster PS. Pharmacokinetics of a novel submicron budesonide dispersion for nebulized delivery in asthma. *Int. J. Pharm.* 365(1–2), 12–17 (2009).

15 Pandey R, Sharma A, Zahoor A, Sharma S, Khuller Gk, Prasad B. Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug-delivery system for experimental tuberculosis. *J. Antimicrob. Chemother.* 52(6), 981–986 (2003).

16 Ostrander KD, Bosch HW, Bondanza DM. An *in-vitro* assessment of a NanoCrystal beclomethasone dipropionate colloidal dispersion via ultrasonic nebulization. *Eur. J. Pharm. Biopharm.* 48(3), 207–215 (1999).

17 Rance RW. Studies of the factors controlling the action of hair sprays: III. The influence of particle velocity and diameter on the capture of particles by arrays of hair fiber *J. Soc. Cosm. Chem.* 25, 545–561 (1974).

18 Timsina MP, Martin GP, Marriott C, Ganderton D, Yianneski M. Drug delivery to the respiratory tract using dry powder inhalers. *Int. J. Pharm.* 101, 1–13 (1994).

19 Crompton GK. Dry powder inhalers: advantages and limitations. *J. Aerosol Med.* 4(3), 151–156 (1991).

20 Finlay WH. *The Mechanics of Inhaled Pharmaceutical Aerosols: an Introduction.* Academic press, London (2001).

21 Atkins PJ. Dry powder inhalers: an overview. *Respir. Care* 50(10), 1304–1312 (2005).

22 Vidgren MT, Karkkainen A, Karjalainen P, Nuutinen J, Paronen TP. *In vitro* and *in vivo* deposition of drug particles inhaled from pressurised aerosol and dry powder inhaler. *Drug Dev. Ind. Pharm.* 14, 2649–2665 (1988).

23 Edwards DA, Hanes J, Caponetti G *et al.* Large porous particles for pulmonary drug delivery. *Science* 276(5320), 1868–1871 (1997).

24 Sung JC, Pulliam BL, Edwards DA. Nanoparticles for drug delivery to the lungs. *Trends Biotechnol.* 25(12), 563–570 (2007).

25 Cheow WS, Chang MW, Hadinoto K. Antibacterial efficacy of inhalable levofloxacin-loaded polymeric nanoparticles against *E. coli* biofilm cells: the effect of antibiotic release profile. *Pharm. Res.* 27(8), 1597–1609 (2010)

26 Suk JS, Lai SK, Boylan NJ, Dawson MR, Boyle MP, Hanes J. Rapid transport of muco-inert nanoparticles in cystic fibrosis sputum treated with *N*-acetyl cysteine. *Nanomedicine (Lond)* 6(2), 365–375 (2011).

27 Roa WH, Azarmi S, Al-Hallak MH, Finlay WH, Magliocco AM, Lobenberg R. Inhalable nanoparticles, a non-invasive approach to treat lung cancer in a mouse model. *J. Control. Release* 150(1), 49–55 (2011)

▪ **First to show the effect of using an active mechanism of nanoparticles (NPs) release in the overall efficacy of inhalable NPs as cancer treatment in an animal model.**

28 Takashima Y, Saito R, Nakajima A *et al.* Spray-drying preparation of microparticles containing cationic PLGA nanospheres as gene carriers for avoiding aggregation of nanospheres. *Int. J. Pharm.* 343(1–2), 262–269 (2007).

29 Sharma A, Sharma S, Khuller GK. Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. *J. Antimicrob. Chemother.* 54(4), 761–766 (2004).

30 Davda J, Labhasetwar V. Characterization of nanoparticle uptake by endothelial cells. *Int. J. Pharm.* 233(1–2), 51–59 (2002).

31 Al-Hallak MH, Sarfraz MK, Azarmi S, Kohan MH, Roa WH, Lobenberg R. Microcalorimetric method to assess phagocytosis: macrophage–nanoparticle interactions. *AAPS J.* 13(1), 20–29 (2010)

32 Muttil P, Prego C, Garcia-Contreras L *et al.* Immunization of guinea pigs with novel hepatitis B antigen as nanoparticle aggregate powders administered by the pulmonary route. *AAPS J.* 12(3), 330–337 (2010)

▪ **One of the first reports of using inhalable NPs as needle-free vaccination.**

33 Muttil P, Pulliam B, Garcia-Contreras L *et al.* Pulmonary immunization of guinea pigs with diphtheria CRM-197 antigen as nanoparticle aggregate dry powders enhance local and systemic immune responses. *AAPS J.* 12(4), 699–707 (2010)

▪ **One of the first reports of using inhalable NPs as needle-free vaccination.**

34 Sung Jc, Padilla DJ, Garcia-Contreras L *et al.* Formulation and pharmacokinetics of self-assembled rifampicin nanoparticle systems for pulmonary delivery. *Pharm. Res.* 26(8), 1847–1855 (2009).

▪ **Only report of using hollow nanoaggregates *in vivo*.**

35 Bruinenberg P, Blanchard JD, Cipolla D, Dayton F, Mudumba S, Gonda I. Inhaled liposomal ciprofloxacin: once a day management of respiratory infections. In: *Respiratory Drug Delivery* 1, 73–82 (2010).

36 Cipolla D, Redelmeier T, Eastman S, Bruinenberg P, Gonda I. Liposomes, niosomes and proniosomes – a critical update of their (commercial) development as inhaled products. In: *Respiratory Drug Delivery* 1, 41–54 (2011).

37 Carvalho TC, Peters JI, Williams RO 3rd: Influence of particle size on regional lung deposition – what evidence is there? *Int. J. Pharm.* 406(1–2), 1–10 (2011)

38 Tsapis N, Bennett D, Jackson B, Weitz DA, Edwards DA. Trojan particles: large porous carriers of nanoparticles for drug delivery. *Proc. Natl Acad. Sci. USA* 99(19), 12001–12005 (2002).

▪ **First report that mentioned the effect of having small Péclet number on the shape of the nanoaggregates.**

39 Kabbaj M, Phillips NC. Anticancer activity of mycobacterial DNA: effect of formulation as chitosan nanoparticles. *J. Drug Target* 9(5), 317–328 (2001).

40 Pilcer G, Amighi K. Formulation strategy and use of excipients in pulmonary drug delivery. *Int. J. Pharm.* 392(1–2), 1–19 (2010).

41 Hinds WC. Aerosol technology: properties, behavior and measurement of airborne particles. Wiley-interscience, NY, USA (1999).

42 Kawakami K, Sumitani C, Yoshihashi Y, Yonemochi E, Terada K. Investigation of the dynamic process during spray-drying to improve aerodynamic performance of inhalation particles. *Int. J. Pharm.* 390(2), 250–259 (2010)

43 Kho K, Cheow WS, Lie RH, Hadinoto K. Aqueous re-dispersibility of spray-dried antibiotic-loaded polycaprolactone nanoparticle aggregates for inhaled anti-biofilm therapy. *Powder Technol.* 203(3), 432–439 (2010).

44 Hadinoto K, Phanapavudhikul P, Kewu Z, Tan BHR. Novel formulation of large hollow nanoparticles aggregates as potential carriers in inhaled delivery of nanoparticulate drugs. *Ind. Eng. Chem. Res.* 45, 3697–3706 (2006).

45 Cheow WS, Li S, Hadinoto K. Spray drying formulation of hollow spherical aggregates of silica nanoparticles by experimental design. *Chem. Eng. Res. Design* 88, 673–685 (2010).

46 Hadinoto K, Phanapavudhikul P, Kewu Z, Tan RB. Dry powder aerosol delivery of large hollow nanoparticulate aggregates as prospective carriers of nanoparticulate drugs: effects of phospholipids. *Int. J. Pharm.* 333(1–2), 187–198 (2007).

▪ **Evaluated the effect of different formulation parameter on the characteristics of dry powder.**

47 Kho K, Hadinoto K. Aqueous re-dispersibility characterization of spray-dried hollow spherical silica nano-aggregates. *Powder Tech.* 198, 354–363 (2010).

48 Hadinoto K, Cheow WS. Hollow spherical nanoparticulate aggregates as potential ultrasound contrast agent: shell thickness characterization. *Drug. Dev. Ind. Pharm.* 35(10), 1167–1179 (2009).

49 Kho K, Hadinoto K. Effects of excipient formulation on the morphology and aqueous re-dispersibility of dry-powder silica nano-aggregates. *Colloids Surf. A: Physicochem. Eng. Aspects* 359, 71–81 (2010).

▪ **Evaluated the effect of using different excipients on enhancing NPs redispersibility.**

50 Tomoda K, Ohkoshi T, Nakajima T, Makino K. Preparation and properties of inhalable nanocomposite particles: effects of the size, weight ratio of the primary nanoparticles in nanocomposite particles and temperature at a spray-dryer inlet upon properties of nanocomposite particles. *Colloids Surf. B: Biointerfaces* 64(1), 70–76 (2008).

51 Tomoda K, Ohkoshi T, Kawai Y, Nishiwaki M, Nakajima T, Makino K. Preparation and properties of inhalable nanocomposite particles: effects of the temperature at a spray-dryer inlet upon the properties of particles. *Colloids Surf. B: Biointerfaces* 61(2), 138–144 (2008).

52 Yamamoto H, Hoshina W, Kurashima H *et al.* Engineering of poly(DL-lactic-co-glycolic acid) nanocomposite particles for dry powder inhalation dosage forms of insulin with the spray-fluidized bed granulating system. *Advanced Powder Technol.* 18(2), 215–228 (2007).

53 Jensen DM, Cun D, Maltesen MJ, Frokjaer S, Nielsen HM, Foged C. Spray drying of siRNA-containing PLGA nanoparticles intended for inhalation. *J. Control. Release* 142(1), 138–145 (2009)

54 Lebhardt T, Roesler S, Uusitalo HP, Kissel T. Surfactant-free redispersible nanoparticles in fast-dissolving composite microcarriers for dry-powder inhalation. *Eur. J. Pharm. Biopharm.*, 78(1), 90–96 (2011)

▪ **Evaluated the effect of using different excipients on enhancing NPs redispersibility.**

55 Wang ZL, Finlay WH, Peppler MS, Sweeney LG. Powder formation by atmospheric spray-freeze-drying. *Powder Tech.* 170(1), 45–52 (2006).

56 Cheow WS, Ng ML, Kho K, Hadinoto K. Spray-freeze-drying production of thermally sensitive polymeric nanoparticle aggregates for inhaled drug delivery: effect of freeze-drying adjuvants. *Int. J. Pharm.* 404(1–2), 289–300 (2010).

- **Showed the feasibility of using spray freeze-drying instead of spray drying for thermally sensitive polymers.**

57 El-Gendy N, Gorman EM, Munson EJ, Berkland C. Budesonide nanoparticle agglomerates as dry powder aerosols with rapid dissolution. *J. Pharm. Sci.* 98(8), 2731–2746 (2009).

58 El-Gendy N, Desai V, Berkland C. Agglomerates of ciprofloxacin nanoparticles yield fine dry powder aerosols. *J. Pharm. Innov.* 5, 79–86 (2010).

59 El-Gendy N, Berkland C. Combination chemotherapeutic dry powder aerosols via controlled nanoparticle agglomeration. *Pharm. Res.* 26(7), 1752–1763 (2009).

60 El-Gendy N, Aillon Kl, Berkland C. Dry powdered aerosols of diatrizoic acid nanoparticle agglomerates as a lung contrast agent. *Int. J. Pharm.* 391(1–2), 305–312 (2010)

61 Plumley C, Gorman EM, El-Gendy N, Bybee CR, Munson EJ, Berkland C. Nifedipine nanoparticle agglomeration as a dry powder aerosol formulation strategy. *Int. J. Pharm.* 369(1–2), 136–143 (2009).

62 Aillon KL, El-Gendy N, Dennis C, Norenberg JP, Mcdonald J, Berkland C. Iodinated NanoClusters as an inhaled computed tomography contrast agent for lung visualization. *Mol. Pharm.* 7(4), 1274–1282 (2010)

63 Tomoda K, Ohkoshi T, Hirota K *et al.* Preparation and properties of inhalable nanocomposite particles for treatment of lung cancer. *Colloids Surf. B: Biointerfaces* 71(2), 177–182 (2009).

- **Evaluated the efficacy of using inhalable NPs in form of nanocomposites for cancer treatment.**

64 Aillon KL, Xie Y, El-Gendy N, Berkland CJ, Forrest ML. Effects of nanomaterial physicochemical properties on *in vivo* toxicity. *Adv. Drug Deliv. Rev.* 61(6), 457–466 (2009).

65 Mizoe T, Ozeki T, Okada H. Preparation of drug nanoparticle-containing microparticles using a 4-fluid nozzle spray drier for oral, pulmonary, and injection dosage forms. *J. Control. Release* 122(1), 10–15 (2007).

66 Sham JO, Zhang Y, Finlay WH, Roa WH, Lobenberg R. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. *Int. J. Pharm.* 269(2), 457–467 (2004).

- **First report to show the use of spray drying to load nanoparticle in microparticle carriers and the effects of different excipients.**

67 Iskandar F, Gradon L, Okuyama K. Control of the morphology of nanostructured particles prepared by the spray drying of a nanoparticle sol. *J. Colloid Interface Sci.* 265(2), 296–303 (2003).

68 Grenha A, Seijo B, Remunan-Lopez C. Microencapsulated chitosan nanoparticles for lung protein delivery. *Eur J. Pharm. Sci.* 25(4–5), 427–437 (2005).

69 Grenha A, Seijo B, Serra C, Remunan-Lopez C. Chitosan nanoparticle-loaded mannitol microspheres: structure and surface characterization. *Biomacromolecules* 8(7), 2072–2079 (2007).

70 Li X, Guo Q, Zheng X *et al.* Preparation of honokiol-loaded chitosan microparticles via spray-drying method intended for pulmonary delivery. *Drug Deliv.* 16(3), 160–166 (2009).

71 Kaye RS, Purewal TS, Alpar HO. Simultaneously manufactured nano-in-micro (SIMANIM) particles for dry-powder modified-release delivery of antibodies. *J. Pharm. Sci.* 98(11), 4055–4068 (2009).

72 Ohashi K, Kabasawa T, Ozeki T, Okada H. 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(1), 19–24 (2009).

73 El-Sherbiny IM, Smyth HD. Biodegradable nano-micro carrier systems for sustained pulmonary drug delivery: (I) self-assembled nanoparticles encapsulated in respirable/swellable semi-IPN microspheres. *Int. J. Pharm.* 395(1–2), 132–141 (2010)

74 Azarmi S, Lobenberg R, Roa WH, Tai S, Finlay WH. Formulation and *in vivo* evaluation of effervescent inhalable carrier particles for pulmonary delivery of nanoparticles. *Drug Dev. Ind. Pharm.* 34(9), 943–947 (2008).

- **Showed using spray freeze-drying technique to formulate effervescent inhalable NPs and the safety of this inhalable powder in animal model.**

75 Niwa T, Shimabara H, Kondo M, Danjo K. Design of porous microparticles with single-micron size by novel spray freeze-drying technique using four-fluid nozzle. *Int. J. Pharm.* 382(1–2), 88–97 (2009).

76 Schiffter H, Condliffe J, Vonhoff S. Spray-freeze-drying of nanosuspensions: the manufacture of insulin particles for needle-free ballistic powder delivery. *J. R. Soc. Interface* 7(4), 483–500 (2010)

77 Abdelwahed W, Degobert G, Fessi H. A pilot study of freeze drying of poly(epsilon-caprolactone) nanocapsules stabilized by poly(vinyl alcohol): formulation and process optimization. *Int. J. Pharm.* 309(1–2), 178–188 (2006).

78 Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process and storage considerations. *Adv. Drug Deliv. Rev.* 58(15), 1688–1713 (2006).

79 Arnold MM, Gorman EM, Schieber LJ, Munson EJ, Berkland C. NanoCipro encapsulation in monodisperse large porous PLGA microparticles. *J. Control. Release* 121(1–2), 100–109 (2007).

80 Ely L, Roa W, Finlay Wh, Lobenberg R: Effervescent dry powder for respiratory drug delivery. *Eur. J. Pharm. Biopharm.* 65(3), 346–353 (2007).

- **First report that discuss the formulation of effervescent inhalable NPs and compares it with other formulations in regard to NP dispersibility.**

81 Al-Hallak MH, Azarmi S, Sun C *et al.* Pulmonary toxicity of polysorbate-80-coated inhalable nanoparticles; *in vitro* and *in vivo* evaluation. *AAPS J.* 12(3), 294–299 (2010)

82 Li YZ, Sun X, Gong T, Liu J, Zuo J, Zhang ZR. Inhalable microparticles as carriers for pulmonary delivery of thymopentin-loaded solid–lipid nanoparticles. *Pharm. Res.* 27(9), 1977–1986 (2010)