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The nanoscale in pulmonary delivery. Part 2: formulation platforms

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This article is the second part of a review on the nanoscale in pulmonary drug delivery. Specifically it summarises and analyses the potential of the different inhalation delivery routes: nebulisers, dry powder inhalers, pressurised metered-dose inhalers, for the delivery of nanoparticles or nanodroplets. Few products and experimental studies have managed to fully exploit the nanoscale in inhalation delivery, although some may unknowingly benefit from it. Nebulisers are the most advanced in using the nanoscale, pressurised metered-dose inhalers require further developments to realise its full potential, and dry powder inhalers are specifically in need of a dry solid nanoparticle generation technique to make it a reality.

Keywords: drug delivery, dry powder inhaler, formulation, inhalation, nano, nanodroplet, nanoparticle, nanoscale, nebuliser, pressurised metered-dose inhaler, pulmonary delivery

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1. Introduction

The first part of this review looked at the deposition, fate, toxicity and effects of the inhalation of nanoparticles. This second paper concentrates on specific delivery technologies.

Although drug delivery via aerosols is an effective and practical method to deliver active drugs to the respiratory tract [1], very few studies have investigated the use of engineered nanoparticles for inhalation, but the relevant published literature (articles, conference abstracts and patents) is reviewed in this paper. The sections on nebulisers, dry powder inhalers (DPIs) and pressurised metered-dose inhalers (pMDIs) deal with the formulation of nanoparticles in these delivery platforms.

This review does not cover nasal delivery, as this is a local delivery technique where the drug does not reach the lungs. Nor does it look at lung ventilation with fluorinated liquids, and the potential to deliver drugs via the respiratory tract in flooded lungs. For the sake of simplicity, the term nanoparticles is used throughout this review to cover solid nanoparticles as well as nano-liquid droplets. When a distinction needs to be made between liquid and solid particles, this is specified. The term 'nano' refers to all particulates, droplets or solid particles in this paper, for which at least one dimension is 1 – 1000 nm.

2. Formulation platforms for the nanoscale in pulmonary delivery

2.1 General considerations on the formulation of nanoparticles for inhaled delivery

The delivery of nanoparticles to the lungs relies on either dry solid particles as in DPIs, suspensions of nanoparticles as in nebulisers and pMDIs, or the formation of nanodroplets from solution. These, in turn, may evaporate to leave nanoparticles residue.

The formulation of dry nanoparticles depends primarily on the availability of solid non-cohesive dry nanoparticles. The main difficulty with their formulation is to prevent particles from aggregating and sintering. The cohesive forces between solid nanoparticles are very strong, and are mostly governed by van der Waals-type interactions, but also electrostatic charges and to some extent mechanical forces. Capillary forces also play a part, generated for instance by hydration films on the surface of the particles.

Formulating nanoparticles in suspension, as in nebulisers and pMDIs, is also frought with challenges. Four major interaction forces can be found in suspensions:

- Repulsive forces (electrostatic and steric)
- Attractive forces (van der Waals)
- Thermal energy (i.e., Brownian motion)
- Gravitational forces (density and size dependent).

The balance of these four forces/energies controls the stability of the suspension. At the micron scale, gravity forces dominate, thermal energy is negligible and so are dispersive attractive forces over long distances (i.e., 1 µm). Therefore micron-sized particles will always migrate to the interface or to the bottom of the delivery device over time. Their ability to aggregate will be controlled by the volume concentration (i.e., average interparticulate distance) and the strength of the repulsive forces. In aqueous suspensions, these can be tailored to prevent particulate aggregation. In non-aqueous liquids, such as in pMDIs, the influence of electrostatic forces is an area of debate. They are generally considered not sufficient to provide stability, and as steric forces are not always available, most non-aqueous suspensions of micron-sized particles are unstable. By reducing the particle size, it is possible to improve the stability of the suspension. Nanosuspensions are almost insensitive to gravity forces, at least over the lifetime of the product; dispersive attractive forces become dominant, but can be overcome, and Brownian motion becomes relevant. The latter can lead to particle aggregation if these are not stabilised appropriately. The theory to describe the stability of nanodispersions derives from the one for microparticles, and only recently have publications appeared dealing with its specificity [2]. However, experience has shown that nanosuspensions can be more stable than microsuspensions, and, therefore, could alleviate some of the issues observed with existing pMDI suspension formulations. Nanoparticles are known to have larger solubility than micron particles. This could have a deleterious effect on suspension formulations because of Ostwald ripening, although methods to inhibit particle growth have been suggested [3]. The suspension of microparticles in aqueous or hydrofluoroalkane (HFA) propellants can lead to the inconsistent aerosolisation of particles (i.e., liquid aerosol droplets do not contain the same amount of drug particles). It is hoped that nanoparticles will remedy this issue in providing a more uniform distribution of solid particles in the liquid droplets.

A further issue for aerosol delivery – in particular with nebulisers and pMDIs – is the likelihood of particle association in aerosol liquid droplets. Gonda *et al.* in the early 80's looked at this issue, and developed a theoretical framework to describe particle aggregation in droplets [4]. The theory, based on statistical filling of aerosol droplets by individual or groups of solid particles, accurately predicted the aggregation of micron-sized particles in aqueous aerosols, but does not seem to be able to achieve the same accuracy for pMDIs [5]. The concentration at which particle aggregation becomes an issue is dependent on the size and concentration of the particles and droplets. At a constant dose, this limit decreases as the nominal particle size decreases, and could become an issue with nanoparticles smaller than 100 nm; however, this remains to be experimentally proven, as the model does not take into account the dynamic nature of the aerosol clouds.

It must be remembered that, in deposition studies, aerosol droplets and particles are highly dynamic systems; '*Often particle size does not remain constant once it reaches the respiratory tract. Volatile aerosols become smaller with evaporation, and hygroscopic aerosols grow bigger with moisture from the respiratory tract*' [6]. Therefore, knowledge of the primary particle size will not be enough to predict deposition, and the formulation of nanoparticles for pulmonary delivery will require knowledge of the dynamics of aerosol behaviour for efficient targeting.

2.2 Nebulisers

Nanoscale delivery by nebulisers is the most advanced of the three delivery platforms that are discussed in this review, and this is set to grow: '*As progress in nanotechnology allows the development of smart drug carrying particles, advanced liquid nebulization is expected to be the delivery mode of choice for these smart particle aerosols*' [6].

Nebulised nanoparticles present distinct advantages over microparticles, as shown in a pharmacokinetics and safety study of nanobudesonide, compared with Pulmicort® Respules® (AstraZeneca) using the Pari LC jet nebuliser. The particles size of the nano-budesonide ranged 75 – 300 nm, compared with Pulmicort Respules (4400 nm). It was found that the nano-budesonide allowed for shorter nebulisation times, and a more rapid rate of drug delivery and/or absorption [7]. In general, nanoparticles have been shown to exhibit a greater stability in the face of extreme forces generated during the nebulisation process, thus eliminating the possibility of drug leakage [8].

Nebulisers are used for the delivery of fine droplets of drugs to the lungs or smaller bronchii, and for this reason have been optimised for aerosol delivery in the upper range of inhalation delivery, 1 – 5 µm [9]. This is true for jet and ultrasonic nebulisers. Yet, it is possible to find examples of nanodroplet nebulised aerosols [10]. The nanoscale is achieved mostly through the nebulisation of nanoparticle suspensions, or by the drying of liquid droplets. Present nebulisers are

capable of generating particles in the nanometer range, which can penetrate the distal regions of the lungs and are, thus, suited for systemic delivery [11,12]. The benefits associated with the nanoscale in nebulisation are a quick onset, longer effects, more regular dosing and equivalent efficiency at lower doses.

The most common way to produce nebulised aerosols is with jet or ultrasonic nebulisers, both of which are presently designed to generate microdroplets, but could be adapted to form nanodroplets.

2.2.1 Jet nebulisers

Jet nebulisers use pressure to break a liquid stream into aerosol droplets [13]. The aerosol droplet size is dependent on the compressed air pressure, with mass median diameters of 2 – 5 µm [14]. These diameters can be achieved once the large droplets (> 10 µm) have been filtered out by impaction on the device surfaces or baffles [9,10]. The droplets formed by jet nebulisation are micron-sized, but nanoparticles could be delivered by filtering out the larger particles and tailoring the liquids' physical properties.

An experiment conducted a few years ago using a colloidal dispersion of beclomethasone dipropionate, where the mean particle diameter was < 400 nm, did demonstrate the feasibility of generating respirable nano-aerosols with a jet nebuliser [15].

2.2.2 Ultrasonic nebulisers

Ultrasonic nebulisers are capable of greater output than air jet nebulisers, and for this reason are used frequently in aerosol therapy. Ultrasonic nebulisers generate aerosols using high-frequency ultrasonic waves. The droplet size distribution is given by Equation 1 [16].

$$D = 0.34 \cdot \left(\frac{8\pi\gamma}{\rho f^2} \right)^{1/3} \quad (1)$$

D is the median droplet diameter, γ is the liquid surface tension, ρ its density and f the vibrating frequency of the piezoelectric crystal used in the device (0.5 – 2.0 MHz on average). For standard liquids such as aqueous solutions (γ = 72 mN/m and ρ = 1 g/cm^3), the droplet size ranges 2.6 – 6.6 µm as the vibrating frequency increases (0.5 to 2.0 MHz). However, if the nebuliser formulation is prepared with fluorinated liquids (with low surface tension [5 mN/m] and high density [1.6 g/ml]) a smaller droplet size range would be generated at the same frequencies: 0.9 – 2.3 µm. This size range could be reduced further by using high-frequency piezoelectric crystals: 5 MHz would be sufficient to form aqueous droplets below 1 µm, and 8 MHz for 0.5-µm droplets.

The production of surfactant nanodroplets of 480 nm via ultrasonic nebulisation has been reported [17], when higher frequencies than those normally found in commercial nebulisers were used (3.3 and 4.0 MHz). In other words, although it is theoretically possible to form nanodroplets with ultrasonic nebulisers, the technology needs to be further developed.

2.2.3 Nanoparticles from evaporated aerosols

Upon evaporation of the carrier liquid, solid nanoparticles can be generated from a solution. Nebuliser formulations contain water with additives, such as solvents and stabilisers, that influence the aerosol droplet size. If the aerosol droplet has time to evaporate before reaching its target, very small particle sizes can be achieved. Evaporation of the solvent will lead to the formation of solid residue particles (made of excipient and drug). An estimate of the final particle size is given by Equation 2.

$$d_p = d_D \left(\frac{C \cdot \rho_D}{\rho_p} \right)^{1/3} \quad (2)$$

d_p is the particle diameter, d_D the aerosol droplet diameter, C is the solution concentration (mass fraction), ρ_D the liquid density and ρ_p the density of the solid particle. From a judicious choice of solute concentration and adjusting of the liquid and particle densities, it is possible to form nanoparticles via nebulisation.

This was achieved by Mikuska [10] with solutions of sodium nitrate and amoniumsulphate. The geometric mean of the dried particles from the solution was 52.1 nm, with a starting concentration of 30 g/l and air flow rates of 78 l/min. Waldrep *et al.* [18] published results on the jet-nebulisation of five different ciclosporine A-DLPC liposomes. A mass median aerodynamic diameter (MMAD) of 820 nm was achieved. Although this can be considered as a success, it was calculated that only 11.6% of the inhaled liposomes would be deposited in the respiratory tract, and the remainder (85.48%) would be exhaled.

2.2.4 Condensation aerosols

Condensation aerosol generation, also called capillary aerosol generators, is another way of generating fine aerosols. These have been studied by academics and industrials alike, and have led to a series of drug delivery platforms developed by Alexza (Staccato™ [201]), Chrysalis (Aria™ [202]) and Coremed (Alveair™ [203]). They work on the principle of producing a highly concentrated vapour of a suitable material in air or an inert gas, and cooling the vapour until it becomes supersaturated and condenses to form a cloud of liquid droplets and/or solid particles [19]. Cooling can be achieved by rapid expansion, thermal conduction or rapid mixing. Natural examples of condensation aerosols are fogs, clouds and exhaust fumes. This is the type of aerosols that was studied by Morawska *et al.* [20]. Parameters influencing

the delivery efficiency of condensation aerosols have been extensively investigated [21].

The application of condensation aerosolisation to nebulisation of therapeutic molecules is relatively recent. Hong *et al.* [22] successfully produced nanoparticles (600 – 900 nm) of benzyl from a 0.7% w/w benzyl in propylene glycol solution with a purpose-designed heated capillary aerosol generator. However, the droplet size was shown to increase with residence time in holding chambers. Gupta *et al.* [19] tested a similar technology with a range of liquids (propylene glycol, ethylene glycol, dipropylene glycol, diethylene glycol, tritethylene glycol, tetraethylene glycol, dimethylsulfoxide, formamide and oleyl alcohol) with the following model drugs: deoxycorticosterone, benzyl and phenyl salicylate. Nanodroplets were formed with 0.5 and 1% w/w deoxycorticosterone in propylene glycol, sizes of 850 nm and 680 nm were reached, respectively. The smallest droplets were formed with pure oleyl alcohol (300 nm). Brown *et al.* [23] used ethanol in a deposition study of capillary-generated aerosol with budesonide. The aerosol droplets dried up to form a dry powder budesonide aerosol with a small median particle size. They reported that 80% of the aerosol was smaller than the final impactor stage cut-off of 180 nm. The corresponding respirable fraction was 94% of the emitted dose, with a medium size in the 50-nm range. Pherphenazine was nebulised by condensation from propylene glycol to yield droplets with MMADs of 400 nm [24]. In their ultrafine particle study of sebacate oil/metal nuclei *in vivo*, Kim and Jaques [25] give a further demonstration of how condensation aerosols can be successfully deposited in the lungs. One of the drawbacks of aerosol condensation is the need to heat the formulation for a brief period at relatively high temperatures (200ºC). This could lead to chemical degradation. However, the brevity of the heating does not prevent molecules as delicate as insulin from being nebulised successfully by condensation [26].

2.2.5 Nebulisation of suspensions

By far the most common way to deliver nanoparticles by nebulisation is through the aerosolisation of suspensions of solid nanoparticles. NanoCyrstals® technology (Elan) developed by Elan/Nanospheres and tested in collaboration with Sheffield Pharmaceutical Inc. is probably the prime example of such a formulation approach [8,101-103]. One of the advantages of a nebulised nanosuspension is the improved content uniformity of the droplets: '*In comparison to microsuspensions, when nebulising nanosuspensions, one logically increases the probability of the drug to leave the nebuliser by means of the aerosol droplets and it is theoretically expected that drug nanoparticles will be more equally distributed into water droplets*' [27]. There is a limited number of patents claiming a specific nanoparticle size range for nebulisation. The primary patent applications and patents are held by Nanosystems, with particles of < 400 nm [8], Elan, with particle size down to < 50 nm [102], Nanosystems, with particles size down to < 100 nm [103] and Pari, with a particle size range of 0.5 – 2.0 µm [104].

2.2.5.1 Preparing nanoparticles for nebulisation

Nanoparticles for nebulisation are manufactured using different techniques.

Niwa *et al.* [28] reported the production of PLGA nanospheres with bafarelin acetate, used as a model peptide drug, using an emulsion phase separation method. They achieved a particle size of 596 nm. *In vitro* testing in aqueous solution by jet nebulisation led to a fine particle fraction (FPF) of 50%.

Kawashima *et al.* [29] reported the preparation of insulin-loaded PLGA nanospheres (400 nm) with a modified emulsion solvent diffusion method in water, followed by freeze drying. Drug loading in the nanoparticles was 46.8% ± 7.01. Eighty percent of the drug was released from the nanospheres at the initial burst, followed by prolonged release of the remaining drug for a few hours in saline at 37°C. The aqueous dispersions of the PLGA nanospheres were nebulised by a sieve-type ultrasonic nebuliser *in vivo* with a FPF of 75%. Blood glucose levels were reduced significantly and the hypoglycemia was prolonged > 48 h, compared with the nebulised aqueous solution of insulin as a reference (6 h). This result was attributed to the sustained release of insulin from the nanospheres deposited widely into the whole lung.

More recently, the same authors reported the preparation of surface-modified DL-lactide/glycolide copolymer nanospheres with chitosan for the pulmonary delivery of elcatonin [30]. The particle size of the elcatonin-loaded nanospheres was 650 nm. The nanosphere suspension was aerosolised with an ultrasonic nebuliser, whereas a reference microsphere suspension could not be aerosolised. After pulmonary administration, the chitosan-modified PLGA nanospheres were more slowly eliminated from the lungs than unmodified PLGA nanospheres. Chitosan-modified PLGA nanospheres loaded with elcatonin reduced blood calcium levels to 80% of the initial calcium concentration and prolonged the pharmacological action to 24 h, which was a significantly longer duration of action than that by chitosan-unmodified nanospheres. These results were attributed to the retention of the nanospheres adhered to the bronchial mucus and lung tissue, and sustained drug release at the adherence site. In addition, the chitosan on the surface of the nanospheres was said to enhance drug absorption.

PLGA nanoparticles encapsulating rifampicin, isoniazid and pyrazinamide (loading 60 – 70%) were prepared with a double emulsion/solvent evaporation technique [31]. Particle size ranged 186 – 290 nm. Aerosolisation by jet nebulisation led to aerosol droplets of 1.88 µm, with no particle aggregation observed upon nebulisation thanks to PVA stabilisation. The benefits included improvements of the half-life mean residence and relative absolute bioavailability

of encapsulated versus free drug. '*The advantage of [the nanoparticles] over inhalable microspheres was clear cut: firstly, it was possible to co-administer multiple antitubercular drugs encapsulated in nanoparticles and secondly, a better therapeutic response was elicited in the case of nanoparticles*' [31].

Melted homogenisation has been used to manufacture 180 – 200-nm gliceryl behenate lipid in an aqueous Tween 80 solution [32]. The particles were labelled with ^{99m}Tc and aerosolised after reconstitution from aqueous solution with a Heyer Ultraaschall ultrasonic nebuliser. No effect of nebulisation was observed on the primary particle size. An important degree of lymphatic uptake was observed, suggesting that nanoparticles could be successfully used for systemic delivery.

The solvent displacement method, developed by Jung *et al.* [33] was used to prepare diethylaminopropyl amine-poly(vinyl alcohol)-grafted-poly(lactide-co-glycolide) negatively charged biodegradable nanospheres. Particle sizes ranged 70 – 250 nm. Particles were nebulised with the Pari jet nebuliser.

Alginate nanoparticles encapsulating isoniazid, rifampicin and pyrazinamide were prepared by gelification of alginate [34]. Nanoparticles were 235.5 nm in diameter, and drug loading was 70 – 90%. Aerosolisation was performed with a jet nebuliser (Medel Aerofamily). It was found that three doses of the nanoparticles administered every 15 days were equally efficient to 45 doses of oral free drugs administered daily. Other advantages included the sustained release of drugs over a longer duration, prevention of premature drug degradation and a reduction in drug toxicity.

Itraconazole nanoparticles were prepared by two methods: evaporative precipitation into aqueous solution, and spray freezing into a liquid. The particles were nebulised with an Aeroneb® Pro nebuliser (vibrating membrane nebuliser; Nektar). High fine-particle fractions of 71 – 85% were recorded, leading to high lung tissue concentrations in murine models [35].

Milling is the simplest and most documented method to form nanoparticles for nebulisation. Jet milling, high-pressure homogenisation or ball milling can be employed. Milling efficiency is dictated by the ability of the mechanical means to produce enough mechanical energy to fragment the solid material, the nature of the grinding medium such as its solid wetting ability, and the ability to prevent agglomeration of the primary particles by the addition of stabilisers. NanoCrystals of beclomethasone dipropiante have been prepared by ball milling a 5% w/w drug suspension in 2.5% w/w PVA over 24 h [15]. Size was reduced to 267 nm. Nebulisation was performed with a Salter lab jet nebuliser. The suspension was physically stable and deemed suitable for nebulisation. The nebulisation process was determined by the aqueous carrier properties and seemed unaffected by the nanoparticles. The respirable fraction was greater with the NanoCrystals (43.5%) than with a suspension of micronised budesonide (7.5%).

Budesonide nanoparticles were also prepared by high-pressure homogenisation with lecithin and tyloxapol. The nanosuspension remained stable over 1 year, with no aggregation or particle size increase, and was not affected by nebulisation with the Pari Inhaler Boy jet nebuliser [36].

2.2.5.2 *Nanoparticles and delivery efficiency and efficacy*

The effect of nanoparticles on delivery efficiency is noticeable. The short-duration ultrasonic nebulisation (Omron Micro-Air® NEU-03, Omron Healthcare) of a concentrated NanoCrystal colloidal dispersion of beclomethasone dipropionate has demonstrated an increased respirable fraction and decreased throat deposition (by Anderson cascade impactor, ACI), in comparison to the commercially available propellant-based product Vanceril® (Schering-Plough) [37]. The respirable fraction ranged 56 – 72% for the nanocrystalline formulation, versus 36% for the propellant system. In addition, the throat deposition as seen in the induction port of the ACI was 9 – 10% of the emitted dose for the novel suspension, compared with 53% for the commercial product. Thus, when used with the appropriate device, a nanocrystalline colloidal suspension of beclomethasone dipropionate affords greater potential drug delivery to the conductive airways of the lung in both quantity, and as a percentage of the emitted dose. Additionally, lower potential throat deposition values were observed, which may retard the development of undesirable side effects, such as candidiasis, when compared with a propellant-based delivery system.

The effect of nebulisation technology on nanoparticle suspension formulations was studied in a comparative study of a range of nebulisers [38]: Pari LC Star Jet nebuliser, Pulmosonic ultrasonic nebuliser and a piezoelectric crystal nebuliser. PVA-g-gPLGA nanoparticles have been prepared with a solvent displacement method with a range of PVA/PLGA ratios. Particle size ranged 93 – 116 nm. The presence of the nanoparticles did not influence the nebulisation process. However, especially with the more hydrophobic particles, some particle aggregation was observed for the jet nebuliser. In this context, ultrasonic nebulisation was recommended.

The efficacy of nebulised nanoparticles of budesonide was studied by Keller *et al.* [39]. Their objective was to identify the device and formulation interactions that should be considered during the formulation of (nano) suspensions for nebulisation. Budesonide submicron suspensions were prepared by high-pressure homogenisation (mean particle size ~ 0.73 µm). The efficacy of the nanoparticles was compared to a micronised Pulmicort suspension (mean particle size ~ 4.4 µm) at the same concentration. Both formulations were nebulised by a Pari LC Plus jet nebuliser, an ultrasonic nebuliser, and a novel perforated vibrating membrane inhaler. Results showed clear differences caused by the interaction of the formulation and the nebuliser. No formulation-related differences could be found regarding the

emitted aerosol, but delivered doses were higher for the nanoparticle suspension than for Pulmicort.

2.2.5.3 *Parameters influencing nanoparticles delivery from nebulisers*

The concentration, as well as surface characteristics, of nanoparticles plays a significant role in determining the physicochemical properties of the suspension and consequently its behaviour during nebulisation [40]. Problems associated with nanosuspension nebulisation have been reported with nanoparticles showing an extreme tendency towards aggregation [41]. Such incidences may not only affect the properties of the aerosols, but also influence the overall dose delivered to the patient, as well as the release kinetics of the formulation once in the alveolar region. McConville *et al.* [35] successfully delivered sub-micron particles (prepared by an array of different methods and containing different surfactants/stabilisers) of itraconazole to the lungs of mice using Aeroneb Pro nebulisers, and found that the lung deposition and clearance in mouse lungs were affected by the composition of the nebulised colloidal suspension.

Nebulisation formulations make extensive use of stabilisers and solubilisers, and their effect on delivery efficiency is documented. Eskandar *et al.* [42] evaluated the effect of Solutol® HS15 (BASF) on aerosol performance of a nanosuspension of ciclosporine A via nebulisation, and found a promising high delivery efficiency. Particle size ranged 500 – 1300 nm, depending on the amount of surfactant (1% w/w was shown to yield the smaller particle size, i.e., the most dispersed system). These were nebulised with a Pari LC Plus jet nebuliser, Multisonic Top ultrasonic nebuliser and Aeroned Profi vibrating membrane nebuliser. Improved drug delivery was shown to occur with an increase in surfactant concentration (FPF increases from 7 to 63%). Buparvaquone, proposed as an alternative treatment for lung infections [27], has been nebulised as a nanosuspension with both jet and ultrasonic nebulisers. The size of the drug particles was reduced to 406 – 473 nm by high-pressure homogenisation with a range of surfactants: Polxamer 188, polyvinyl alcohol and glycerol. The suspensions were nebulised with a selection of nebulisers: Respi-jet Kendall, Pari Turbo Boy system, the Multisonic and Omron U1 nebulisers. Some particle agglomeration was observed depending on the amount of surfactant in the formulation and the type of nebuliser. Ultrasonic nebulisers for instance seemed to reduce the occurrence of agglomeration.

2.3 Dry powder inhalers

Although nanoparticles have been delivered extensively to the lung using nebulisers, the use of nanoparticles in DPIs is still a relatively unchartered area.

The patent scene for DPIs is relatively simple. Only a few patents with direct reference to the use of nanoparticles in DPIs have been found in a search of the 10 years [105-109]. These are patents that claim particular size ranges, although not with associated benefits. The size range covered is invariably > 500 nm, except for two patent applications: on nanocell particles designed for controlled release [110,43] with a size range of 80 – 120 nm, and not exclusively for inhalation, and the Nanosystem™ (Elan) patent application [103] reviewed earlier with claims for size ranges '*less than about 50 nm*.' This paucity of intellectual properties coverage leaves a wealth of opportunities for future inhalation product development.

The rather sparse application of nanotechnology to DPI delivery can be seen as a direct consequence of the difficulty of generating dry solid non-cohesive stable nanoparticles. So far, this challenge has not been fully met.

2.3.1 *Trojan particles*

Of the few articles in the literature that cover nanoparticles in DPIs, most tend to expand on the theme of Trojan particles [44]. These are micronised matrix particles containing nanoparticles.

Ostrander *et al.* [45] have carried out one of the earlier works on Trojan particles, where, by means of a novel formulation technology, nano-budesonide was first milled to the nanoscale, and then spray dried (D_{v50} = 166 nm after wet milling, and D_{v50} = 1.35 µm after spray drying), and successfully delivered the particles to the lung using a DPI. Tsapis *et al.* [46], Sham *et al.* [47] and Huber and Wirth [48] have also covered the use of Trojan particles delivered via DPIs. Nanoparticles of gelatin (242 nm) and polybutylcyanoacrylate (173 nm) were thus encased in a lactose matrix and spray dried from water to form microparticles (2.6 µm), with a loading capacity of up to 69%. These were tested in a proprietary DPI device and showed acceptable delivery properties. The method of preparation of these particles is the subject of a recent patent application [109]. The ultimate example of a Trojan particle is provided by the NanoCrystal technology [103,45]. These particles, prepared by spray drying after milling, were tested in the Clickhaler™ (Innovata Biomed), and showed the expected delivery performance for micronised particle delivery. More recently, Grenha *et al.* [49] succeeded in encapsulating protein with chitosan nanoparticles using mannitol and lactose. Cook *et al.* [50] described a process for generating sustained release nanoparticles for pulmonary drug delivery. These particles were based on terbutaline sulphate, salbutamol sulphate and ipatropium bromide. High-purity nanoparticles were entrapped within hydrophobic microspheres using a spray-drying approach. These were delivered using a PennCentury Model DP-4 insufflator. The size of the primary nanoparticles was 238 nm, and the respirable particles were in the expected micrometer range for inhalation delivery. A range of surfactants was used to stabilise the suspension. High FPFs and good controlled release properties are reported.

2.3.2 *Particles with nano-features*

Another concept is particle surface modification with nano-features. Kawashima *et al.* [51] coated panlukast hydrate microparticles (2.1 μm) with hydroxy propyl methyl cellulose phthalate nanoparticles. The particulate aggregates were prepared by an emulsion-solvent diffusion technique followed by freeze or spray drying. These particles, with increased roughness and hydrophilicity, were shown to have improved delivery efficiency when delivered with the Spinhaler® (Fisons). Delivery improvement via surface modifications was also achieved by Chew and Chan [52] who reported the preparation of serum albumin particles with nanoscale surface features (described as smooth and wrinkled), leading to much improved FPFs. A further study by Niwa *et al.* [28] prepared nafarelin acetate–lactide/glycolide copolymer nanoparticles. These were tested in the Spinhaler, and the modification of the particle surface properties (rendered hydrophobic) is said to be responsible for the improved delivery performance of the formulation. Surface modification can also be achieved with ultra-thin coatings as demonstrated by Talton *et al.* [53] who deposited 100 nm "nanoclusters" of PLGA onto dry budesonide and triamincinolone acetonide (500 nm – 1000 μm) powders.

2.3.3 *Strawberry particles*

A new concept in DPI formulation was presented at the last annual meeting of the Aerosol Society (Drug Delivery to the Lungs XVI, Edinburgh December 2005).

DPI formulations are often based on a carrier–drug system, where the carriers (mostly lactose) are large particles (50 – 100 μm) onto which smaller active particles (1 – 3 μm) are bound via physical absorption. These 'strawberry' particle mixes are easily handled and lead to high FPFs. Mykhaylova and Urbanetz [54] from Dusseldorf University have turned this concept round by coating fine drug particles (1 – 3 μm) with carrier particles in the nanometer range. They suggested that the nanoparticles act as spacers or surface rougheners and contribute to decreasing interparticulate adhesion between the microparticles. A blend of 12% Aerosil® (Degussa AG) nanoparticles with 88% micronised lactose were tested in this way, and aerosol delivery results were generated with a DPI system. Delivery performance was acceptable for a non-optimised system, with a FPF of 30%. This was the first time such a particulate concept was introduced to the field of aerosol delivery, although it has been discussed in material science before [55]. The particles can be coated or simply mixed. This is known in colloid science as hetero-stabilisation, due to the heterogenous nature of the particle size distribution. The difficulty in coating the microparticles was resolved in this case with an electrostatic mixing of micron-sized lactose with nanosized Aerosil in liquid nitrogen. The resultant powder was less cohesive than the non-coated lactose and more easily fluidised.

2.4 Pressurised metered-dose inhalers

pMDIs can be formulated in two ways: as solutions or suspensions. Both types of formulation can be in the nanoscale. Solution formulations can be tailored to form nanodroplets on aerosolisation (with the help of an appropriate device), and suspension formulations can be prepared to yield nanoparticles.

The effect of ultrafine particles on the efficacy of HFA pMDIs was alluded to briefly in the introduction and the toxicology sections (see Part 1 of the review).

There is a growing body of evidence that the nanodroplets and nanoparticles of HFA pMDIs are responsible for much of their efficacy and side effect [56,57]. For instance, HFA–beclometasone dipropionate is said to manifest its clinical benefits at a lower dose compared with conventional inhaled corticosteroids because of a greater lung deposition, particularly to the peripheral airways. This may be a consequence of its smaller particle size and greater FPF [58]. This efficiency increase was also observed and reported recently for Qvar® (Riker Labs) [57]. A similar effect was observed in a comparative study between Qvar Autohaler administered at 400 μg/day versus Pulmicort Turbuhaler® (800 μg/day) [56]: Qvar was more efficacious than Pulmicort at half the dose. It is not improbable that this lower dosing is made possible thanks to the fine droplets of the Qvar formulation platform. The inhalers in these studies all have FPFs and MMADs in the upper respirable ranges for inhalation (i.e., 1 – 5 μm), but the size of beneficial fine particles is < 1 μm. However, these are not characterised on a routine basis.

One of the reasons for the lack of data relating size and efficacy below a size of 1 μm lies in the difficulty in characterising aerosol nanodroplets and nanoparticles. The only thorough study so far of the relationship between nanoparticles and delivery efficiency was published by Crampton *et al.* [56]. They studied five different commercial solution and suspension HFA pMDIs, and found that all the HFA devices tested '*yielded high numbers of fine (< 1000 nm) and ultrafine particles (< 100 nm)*.' The study was performed with a modified electrical low-pressure impactor coupled with a scanning mobility particle sizer. Qvar and flixotide 125 mg for instance had an MMAD equal to 54 nm, for Ventoli® (GlaxoSmithKline) it was 50 nm, and for Salbulin it was 85 nm. They contended that one of the reasons the role of fine particles was not understood properly, was that ACIs and next-generation impactors are not designed to sample ultrafine aerosols and, therefore, cannot account for them. Were to be these quantified, the improvements observed with fine and ultrafine aerosols could be explained.

2.4.1 *Solution pressurised metered-dose inhalers*

That HFA solution formulations can yield large numbers of fine has been known for a little while, but reported only recently [59,60]. Pure propellant aerosol size distributions are bimodal, with a peak at 600 nm and another at 1 – 2 μm,

depending on the hardware used. Therefore, solution pMDIs will always deliver nanodroplets. These can be modulated with the solute content, such as drug or excipients. This is the case with the Qvar pMDI. The only HFA product that claims to deliver superfine (i.e., smaller than 1 µm) droplets is Chiesi formoterol HFA formulation [111].

Microemulsions can also be prepared as an alternative to true solutions. Some examples exist of the preparation of microemulsions in HFAs; however, their preparation is limited due to the lack of solubility of surfactants in HFAs. Microemulsions are seen as a way to solubilise water-soluble drugs in water-in-HFA microemulsions. The earliest example of an HFA microemulsion is provided by Butz *et al.* [61]. They prepared a double microemulsion: a water in perfluorooctylbromide microemulsion was prepared and stabilised with a fluorinated dimorpholinophosphate. This microemulsion is then dispersed in HFAs. The most stable formulations were obtained for emulsion/propellant ratios of 2/3 – 3/2 in HFA 227. The size of the water emulsion in perfluorooctylbromide was 58 – 200 nm. The uniformity of delivery of the microemulsion was assessed by weighing fired shots. The water content was assessed, and a test on the size of the water droplets was also performed: these did not reveal any destabilisation brought about by the aerosolisation. However, no *in vivo* or *in vitro* delivery efficiency data is available. In a similar fashion, Patel *et al.* [62] discovered a stable water-in-HFA 134a microemulsion prepared with a fluorinated non-ionic oxyethylene glycol surfactant upon the addition of a short chain alcohol (such as ethanol, propanol). The droplet had an average radius ~ 2 nm, as measured by small-angle neutron scattering. Steytler *et al.* [63] prepared a water-in-HFA 134 microemulsion with the aid of 1H,1H,5H octafluoro-*N*-pentyl sodium sulfosuccinate (up to w > 60 [w is the molar ratio of water/surfactant]), and with perfluoropoly propylene oxide carboxylate. The microemulsions prepared were sized with dynamic light scattering and small-angle scattering, and yielded sizes of 2 – 7 nm.

Other attempts [64] to form microemulsions have led to fluorinated liquids in water microemulsions with the addition of a range of oils (notably triglycerides) and could be used as a starting point to further investigations on the formation of microemulsions in HFAs. Although microemulsions can be prepared in HFAs, their success is not proven and will depend on their drug loading ability, as well as their aerosolisation properties.

2.4.2 Nanoparticles and pressurised metered-dose inhalers

As most suspension pMDIs are prepared with particles tailored within the micrometer range, the likelihood of nanoparticles exiting from the aerosols is low; however it has been observed [56]. This could be due to either small solid particles (particle size distributions are rarely unimodal), or drug solubilised in the propellant carrier and aerosolised in nanodroplets. An alternative way to deliver nanoparticles from suspension HFA pMDIs would be to formulate solid nanoparticles in HFAs, which, upon aerosolisation, would populate micron-sized aerosol droplets. This depends on the manufacturing of solid dry nanoparticles, as for DPIs. This is proving to be the limiting factor in bringing the nanoscale to inhalation delivery.

To the present authors' knowledge, only one commercial supplier of dry active pharmaceutical ingredient nanoparticles: Nanomaterials, from Singapore. With this technique, the crystallisation phenomenon is enhanced by increasing the local gravity field during solution mixing to trigger precipitation [65]. This method, known as high gravity reactive precipitation, produces inorganic nanoparticles with sizes ranging from 1 to 300 nm depending on the material and processing conditions. Its adaptation to treat organic materials is recent, and is the most promising of all processing techniques reviewed so far [66]; the example provided was based on benzoic acid. The key element of high gravity reactive precipitation is a rotating packed bed, where two streams of liquids meet and react to form nanoparticles [67]. The high gravity generated in the packed bed due to their rotations at high speed is the key to nanoprecipitation. This is equivalent to rapid micro-mixing of the reactants to enhance nucleation while suppressing crystal growth. Benzoic acid was precipitated as nanoparticles as fine as 10 nm.

Supercritical fluids can also extract particles from emulsions. Griseosulvin and megestrol nanoparticles have been produced from a water-organic liquid microemulsion by extraction with CO_2 [68]. The process led to the formation of a suspension of nanoparticles ranging from 60 to 978 nm. Nanoparticle extraction from a microemulsion was more successful with snap and freeze drying. Dry spherical nanoparticles (< 300 nm) of salbutamol sulphate were prepared from a double emulsion by Dickinson *et al.* [69]. These were suspended in HFA 227 and showed very good delivery properties (FPF > 58%). These are the only dry nanoparticles patented for HFA pMDI delivery [112].

Unlike nebulisers, it is not easy to mechanically reduce the size of solid particles *in situ*, as is the case in pressurised HFAs. Milling under pressure is difficult, but has been carried out [113,114]. Nanosystems reported the milling of budesonide in HFAs to the nanoscale in the presence of stabilisers (PEG and PVP) for the purpose of formulating in HFAs [102,103]. The size range claimed is < 1000 nm, with a provision for particles < 50 nm. The pressure milling idea was also suggested by Dupont de Nemours in recent patent applications [115,116].

2.4.3 Particle tailoring

Another way to introduce a nanodimension in pMDIs is via particle tailoring. Trojan particles can be formulated in HFAs, micron-sized particles can be coated with nano polymeric films to improve their stability, or their surface

modified with nano-features. Trojan particles are prepared in the same way as DPIs and nebulisers. WO0178689 claims their particular application to HFA pMDIs, with a size range between '*1 and up to but not including 1000 nm*' [117]. The method of preparation is similar to the one published by the inventors a few years later [50] (i.e., post-processing with a spray-drying method for instance. The studies that led to the filing of the application were eventually published with salbutamol sulphate nanoparticles (300 nm) as an example [69]. The surface of particles can be modified with nano films: WO99384939 [118] describes a suspension formulation in HFAs with a membrane-forming lipid and surfactant as additives. A specific size range for the particles is claimed: 0.1 – 10.0 µm.

2.4.4 *Secondary particulate formulations*

Secondary particulate systems have been known to provide good stability to HFA suspension and enhance delivery characteristics [70]. Briefly, these consist in the hetero-stabilisation of one particle population of a defined size by another of a different size, generally lower (similar to the strawberry particles described in the DPI section). This concept has been known for some time in colloid science, and is well documented [71]. The smaller particles act as stabilisers for the larger one and help to reduce dispersive forces. This formulation concept is similar to stabilisation with bulking agents [72]. Taylor *et al.* [73] have thus prepared L-leucine-fluticasone propionate suspension in the micrometer range. The leucine particles (38 – 125 µm) act as carriers for the drug particles (5 µm). The suspensions were shown to be very stable and their aerosolisation properties satisfactory for inhalation.

It is easy to see how this type of formulation could be adapted to the nanoscale if solid dry nanoparticles were available: micron-sized particles (3 µm) could be stabilised by nanoparticles. However, only recently has this been achieved. 3M filed a patent application where inorganic nanoparticles are used to stabilise micronised drug particles [119]. The tightest size range claimed is '*less than about 100 nm*.'

3. Conclusion

Nanoparticles can be effectively delivered to the lungs via nebulisation, either as droplets of liquid of solid nanoparticles. Nebulised nanoparticles have been shown to have several advantages over micronised material, among which, enhanced effects, prolonged release and improved targetting. The interplay between formulation and device is well documented, and although no commercial product makes explicit use of it so far, the use of nanoparticles in nebulisation is set to grow.

The review of the nanoscale for the three major inhalation delivery platforms: nebulisers, DPIs and pMDIs, has revealed the differences in their development. These findings, and how the nanoscale can be exploited for the different inhalation technologies have been summarised in **Table 1**.

Nebulisers are the most advanced of the three delivery platforms. Nanoparticles and nanodroplets have been successfully formulated and delivered with nebulisers. Progress needs to be made with the level of output, but the nanoscale is a reality with nebulisers.

The nanoparticles–DPI landscape is rather sparse. No products on the market knowledgebly makes use of the nanoscale, no published patents can be found – bar one covering sizes below 500 nm – and no papers in the literature have fully explored the issue. The reason for this dearth of inventions may be found in the difficulty of processing active pharmaceutical powders to form stable dry nanoparticles. This is a challenge that needs to be addressed, and much might be learned from the chemical industries, where the nano-processing of dyes and inorganic compounds is common practice.

Nanodroplet generation in pMDIs is possible from solution pMDIs, and may account for a large part of their clinical efficacy. However, to consistently and efficiently generate nanodroplets from HFA pMDIs, a redesign of the existing devices is necessary. As for suspension pMDIs, in addition to new device concepts, nanoparticles need to be processed. This could be done *in situ*, as is the case with pressure milling, or *ex situ* through the production of dry solid stable crystalline nanoparticles, as for DPI particles. The challenge for pMDI nanodelivery is therefore twofold: device design and nanoparticle processing, but the outlook is more hopeful than for DPIs.

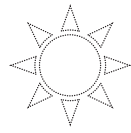
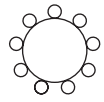
4. Expert opinion

It is possible to deliver and formulate nanoparticles to the lungs, and for these particles to have a local or systemic effect. As yet, this opportunity has not been fully exploited, and, thus, offers formulation scientists a rare opportunity to develop new concepts in inhalation delivery.

The present principal limitation to fully exploiting the nanoscale is the lack of reliable large-scale manufacturing methods to prepare dry solid and stable crystalline nanoparticles. This is certainly limiting the development of a nanoscale DPI and of a suitable suspension pMDI, and has some impact in nebulisers. Some technologies exist, such as high-gravity precipitation, but these have yet to be taken to a commercial large scale basis. The pharmaceutical industry should look at other industries to adapt their processes to produce nanoparticles. For instance, nanoscale gold, silver or titanium dioxide have long been prepared in the dry state.

The application of the nanoscale to inhalation delivery is forecasted to herald new treatment avenues, such as improved controlled release, or even become the route

Table 1. A summary of how the nanodimension can be, and is presently used in inhalation technologies.

Technology	Comment
Nebulisation	
	Sizes achieved (nm):
Jet nebulisation	400.0 (liquid droplets) 52.1 (solid residue) 93.0 (from a suspension formulation)
Ultrasonic nebulisation	480
Condensation nebulisation	50
Electro Hydro Dynamic nebulisation	2 – 10
DPI	
Trojan particles	
	Drug core coated with excipient
Surface modifications	
	Drug core with chemical or physical surface modifications
Strawberries particles – hetero-stabilisation	
	Microparticle surrounded by nanoparticles. Drug and excipients can be swapped
pMDI	
Solution pMDI	Pure solutions, formation of nanoparticles upon aerosolisation
	Microemulsions, nanoparticles already contained in the bulk
Suspension pMDI	Pickering emulsions (emulsion stabilised by nanoparticles)
	Hetero-stabilisation (use of secondary particles)
	Nanoparticles stabilised by surfactants
	Trojan particles

DPI: Dry powder inhaler; pMDI: Pressurised metered-dose inhaler.

of choice to deliver proteins. A range of publications are available on exploratory nanoscale formulations, aiming at leveraging the advantages of the nanoscale for specific lung treatments.

For instance, nanoparticles have been foreseen as an efficient way to develop controlled release delivery systems for the lungs. Videira *et al.* [32] provide a very adequate summary of the importance of controlled release in inhalation: '*Aerosolised particulate carriers may also act as a reservoir in the lungs, providing a sustained release effect with therapeutic interest in many clinical situations that need chronic dosing, such as treatment of lung cancer, chronic pulmonary infections, immunosupression in lung transplants and asthma*.'

Nanoparticles are also said to enable formulations for new inhalation therapies, such as protein delivery, gene therapy, cancer treatment, anti-tuberculosis treatment and systemic delivery. There has been great interest in the pharmaceutical field of pulmonary delivery of insulin and proteins in general, and nanoparticles seem to promise new ways of delivering them.

Elcatonin encapsulated in chitosan-modified nanospheres has demonstrated an increase in its bioactivity compared with non-modified nanoparticles or solution formulations [74]. The nanospheres also demonstrated a slower elimination rate – approximately half that observed with unmodified nanospheres. Blood calcium reduction lasting up to 8 h was increased to over 24 h with the modified nanoparticles. These modified nanospheres (when aerosolised) were said to adhere to the mucus in the trachea (tests performed on guinea pigs), as a result of the mucoadhesive properties of

chitosan, and release the drug over long periods of time, hence demonstrating the possibility to control drug release via nanoparticles.

Insulin nanoparticles, prepared with polybutylcyanoacrylate and delivered in the trachea of guinea pigs, prolonged hypoglycaemic effects from 7 h to 11 h at a low dose, and from 12 h to 20 h at a high dose compared with insulin solutions [75].

Similarly, nanoparticles are being considered in treatments for tuberculosis [31,76], and nanoparticles have been evaluated on other occasions for gene therapy [77].

Therefore, it is our opinion that the nanoscale in inhalation delivery is an open field for inventions and new products, and if inhalation delivery research is looking for a new breath of creativity, after the quantum leaps in porous particles, new excipients for HFAs and systemic delivery of insulin, nanoparticles should provide it.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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