Nanocarriers made from non-ionic surfactants or natural polymers for pulmonary drug delivery.

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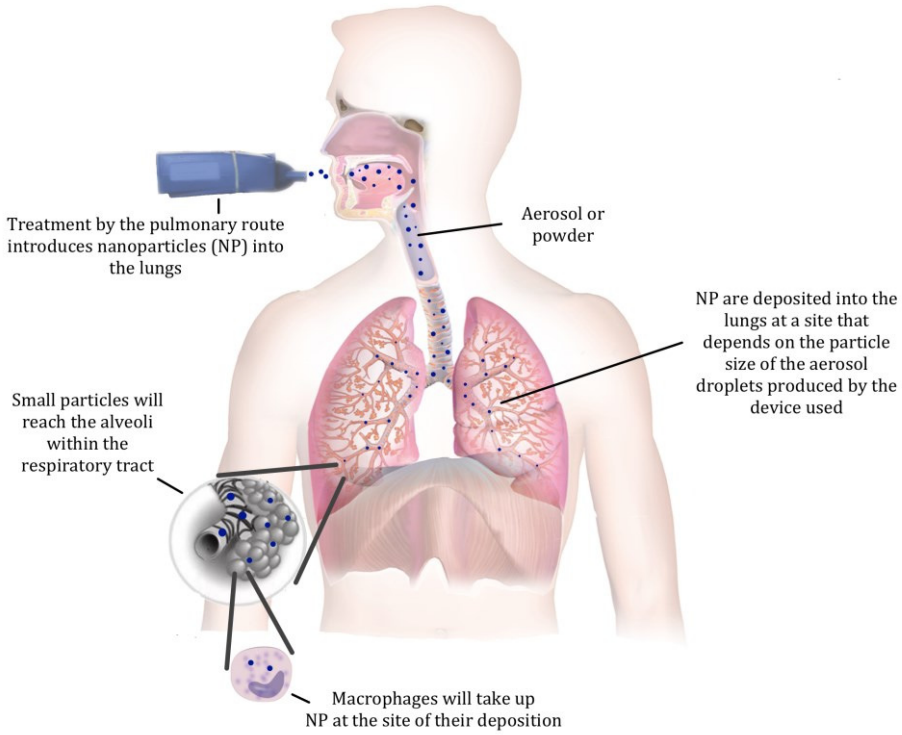
Graphical abstract



Abstract

Treatment by the pulmonary route can be used for administration of drugs that act locally in the lungs (e.g. treatment of lung cancer, chronic obstructive pulmonary disease, asthma) or non-invasive administration of drugs that act systemically. The potential of drug delivery systems formed from non-ionic surfactants or natural products i.e. proteins and polysaccharides for pulmonary delivery are discussed.

Introduction

Treatment by inhalation can be used to deliver drugs directly to the lungs to treat conditions such as lung cancer, tuberculosis, cystic fibrosis or asthma; or as a means of treating systemic conditions such as diabetes or analgesia and the pulmonary drug delivery market is estimated to be worth £28.7million by 2019 [1]. Treatment by inhalation is more patient friendly than parenteral injection. It also allows the drug to avoid the first pass metabolism which occurs in the liver, and the concentration of metabolising enzymes such as CYP450 is lower in the lungs compared to other organs [2]. However successful drug delivery via this route requires production of a drug formulation that is effective, stable and safe but it must also be suitable for inhalation and have the correct characteristics to reach the appropriate site within the lungs. Additionally a drug must evade the innate defense mechanisms present in the lungs, such as mucociliary clearance and macrophage uptake before it reaches its site of action [3]. The lungs are primarily designed for gas exchange and have a symmetric dichotomously branching structure (Figure 1). The upper respiratory tract consists of the nasal cavity and pharynx and the lower respiratory tract consists of the larynx, trachea, bronchi, bronchioles and alveoli [4]. The surface area of the lungs increases from 2 m^2 in the upper respiratory tract to 103 m^2 in the lower airways. And cell thickness decreases from 60 µm in the bronchi to 0.1 µm in the alveoli. Aerosol particle size is a key parameter in defining the drug deposition within the lungs. Aerosols with a small particle size (< 2 µm) are distributed in the peripheral airways, whereas larger aerosols (> 5 µm) are deposited in the central area of the lungs. Particles are deposited in the lungs by inertial impaction, gravitational sedimentation or Brownian diffusion. Inertial impaction predominates, as large particles (> 10 µm) cannot follow the fast

airflow in the conducting airways, and impact into the walls of the upper tracheobronchial region. Particles that impact on the mucus barrier are then cleared by the mucociliary escalator system, where ciliated epithelium moves mucus entrapped particles towards the pharynx where they are removed by macrophages or expectorated [5]. Smaller aerosol particles (< 5 μm) sediment in the bronchi and bronchioles or reach the alveoli, where they are exposed to Brownian diffusion as the air velocity is negligible within the alveoli. Thus aerosol particles between 1 and 5 μm can reach the lower respiratory system [6]. Very small particles (< 0.1 μm) cannot be deposited in the airways as they are breathed out easily [7]. The optimal site of aerosol deposition depends on the particular application. For instance, the β_2 agonist salbutamol should be delivered to the peripheral areas of the lungs as β_2 receptors are located in the bronchi and bronchioles [8] whereas the muscarinic antagonist ipratropium bromide, should be deposited in the conducting airways as muscarinic M3 receptors are predominant in the conducting airways [9]. For the treatment of systemic diseases, the inhaled drug should be deposited in the peripheral areas that are rich in alveoli, where systemic absorption is facilitated by the thin alveolar-vascular barrier [10]. Drug deposition can also be influenced by pathological bronchoconstriction, inflammation or airway obstruction, leading to uneven or central deposition of the drug formulation within the respiratory tract [11, 12].

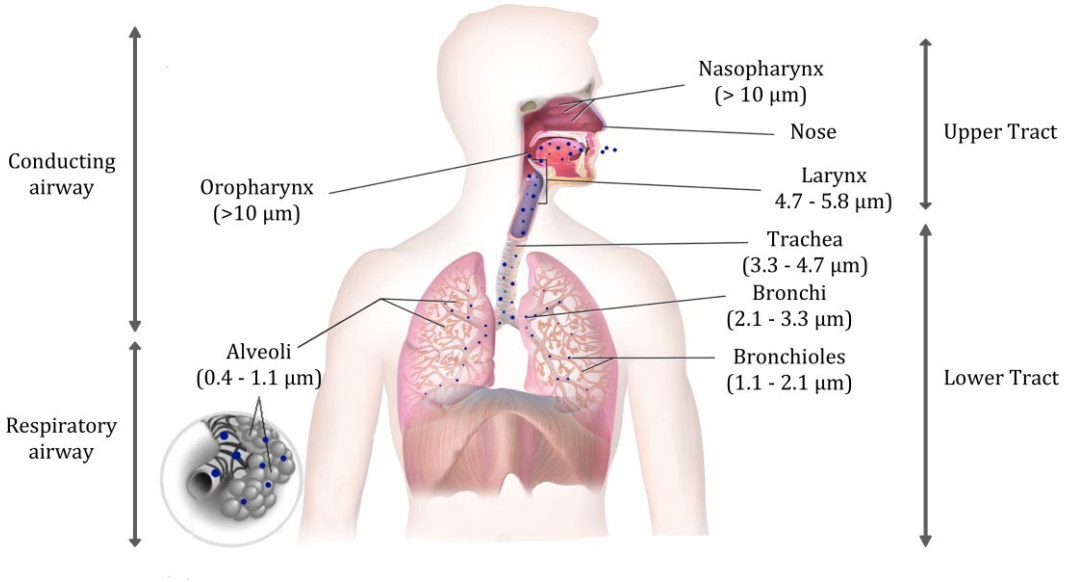


Figure 1 Areas of the respiratory system based on physiological characteristics or anatomical parts. Inhaled aerosols are deposited in different areas of the respiratory system according to their droplet size (adapted [10,13,14]).

Influence of the inhalation device

The development of an effective inhaled therapy depends on the pharmacology of the active ingredients, its aerosolisation characteristics and the efficiency of the aerosol generating device e.g. pressurised metered-dose inhaler, dry powder inhaler or nebuliser. There are a wide range of pulmonary devices [15] and new generation inhalers are very efficient at producing aerosols with well characterised properties. Thus has lead to more effective targeting of the nebulised drug formulation to the lungs, which can lead to the development of a specialised drug-device combination for a particular application. Some inhalers have added adaptions to increase 'ease of use', which is of particular importance for therapies directed towards patients with an older patient profile, or the ability to obtain data on the dose delivered to the patient [16].

It is possible to measure the aerosol particle size *in vitro* using an impactor, where an aerosol hits a flat surface and is separates into different size ranges depending on where it lands in the impactor or impingers. The newest impactor described in the European Pharmacopoeia is the next generation impactor (NGI), which is the only

impactor that works horizontally and collects the aerosol droplets on cups of different cut-off diameter. It has seven stages and a micro orifice collector. The European Pharmacopoeia describes one twin and three multistage apparatus [17]. The twin impinger is operated at a flow rate of 60 L/min and has a cut-off diameter of 6.4 µm. This meaning that particles found in the second stage correspond to the respirable portion (< 6.4 µm). The multi-stage liquid impinger (MSLI), can be used at different air flows, and has a mouth piece attached to the aerosol device, 4 stages and filter paper in the fifth compartment collects the remaining particles. The advantage of the MSLI over other impactors is the presence of solvent in each collection stage. This is important, as it avoids the re-entrainment phenomena of aerosol droplets being reincorporated into the airflow, particularly with DPIs. The data obtained using any of the multi-stage impingers or impactors can be used to calculate the aerodynamic aerosol size distribution of an aerosolised formulation. The mass median aerodynamic diameter (MMAD) of an active ingredient is the diameter at which 50 % of the particles by mass are bigger than the other 50 %. The MMAD is calculated when the log-normal distribution of the mass-weighted data is assumed by plotting a base ten logarithm cut-off diameter against cumulative percentage undersize [18]. The distribution of the particles in the apparatus is generally described by the geometric standard deviation (GSD) and a GSD closer to the one indicates a mono-distributed aerosol size. Fine particle fraction (FPF < 5 µm) is the fraction of the aerosol mass contained in particles with an aerodynamic diameter smaller than 5 µm and larger than 0.98 µm. Achieving a low MMAD and a GSD close to the one indicates a fine aerosol size with a tight size distribution. However, the aerodynamic aerosol size distribution calculated with impactor techniques can only be classified into a small number of size ranges depending on the number of stages of the apparatus. For example, the twin impactor possesses one cut-off diameter and the NGI seven. Electrostatic charge and fine particle adhesion on the walls of the apparatus and losses between stages may also disturb particle collection. Another way to characterize aerosol characteristics is to use laser diffraction techniques. These are easy-to-use and can analyse particles over a broad size range. In addition, the measurement is fast, non-flow dependent and possess automatic data recording. A unique characteristic of laser diffraction methods is being able to carry out time measurements of the cloud distribution [19] and analyse

multi-modal drop size aerosol distribution [20]. However, low particle concentration may lead to low laser obstruction so the aerosol cloud may not be able to be measured. Laser diffraction does not measure aerodynamic diameter. Instead, it measures geometric diameter in terms of mass median diameter (MMD). This value does not consider particle density and assumes that particles are spherical. Pilcer *et al.* [21] compared the values of respiratory fraction obtained with MSLI and the NGI with the values acquired from laser diffraction techniques when powder formulations were aerosolised. The data obtained from the impactor and the laser diffraction differed. However, they found a good correlation factor between both aerodynamic diameter and geometric diameter results.

Animal studies

Animal models have been extensible used to investigate the effect of inhaled drug therapies [22, 23]. However, the physiology of the human airways is spherical with symmetric branching, and these features are not present in other species [24]. Moreover, breathing pattern and obligate nose breathing in rodents are also distinct from humans [12, 25, 26]. Despite those differences, Schlesinger [27] demonstrated a similar relationship between aerosol size and lung deposition in humans and animals such as are dogs, rats, guinea pigs, hamsters and mice. However, alveolar distal impaction reached a peak between 2 and 4 µm in humans but experimental animals have a peak nearer 1 µm. Some researchers have used mathematic models to study the aerosol distribution in the airways as an alternative to *in vivo* models [28] but these methods are highly complex and may not reflect all the conditions that occur *in vivo.* Imaging techniques, have been used to give a more accurate picture of drug deposition e.g. radiolabelled inhaled drugs detected by scintigraphic studies [29], positron emission tomography imaging [30], magnetic resonance imaging [31] or fluorescent imaging [32]. It is now possible to use a combination of imaging methods to improve the signal and *in vivo* detection of a system. For example using quantum dots, which emit a strong fluorescent signal that is not photobleached, coupled with MRI provides better visualisation than MRI alone [33]. In our studies we have used luciferin loaded non-ionic surfactant vesicles to show that the drug delivery system

(DDS) significantly improves *in vivo* targeting of luciferin to luciferase-expressing cells within the body.

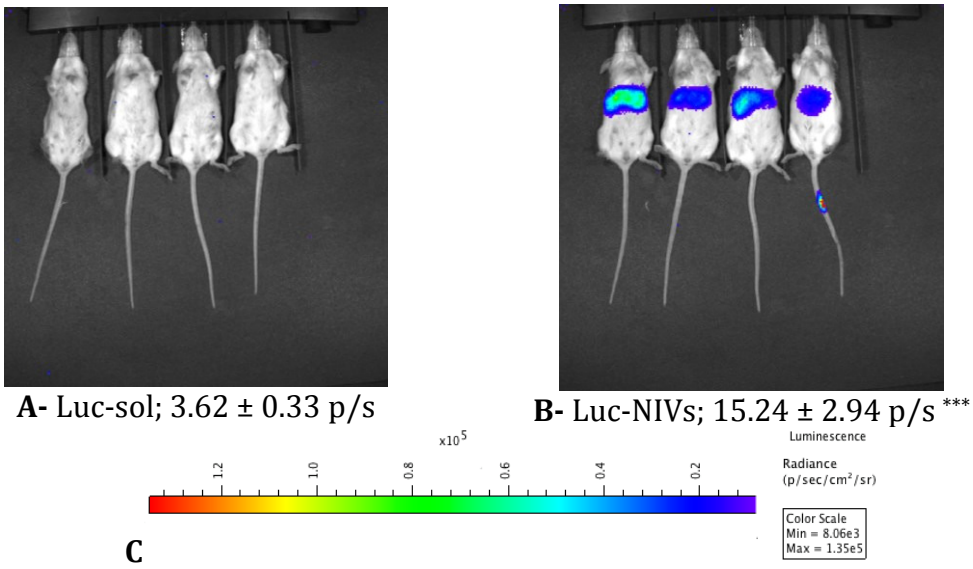


Figure 2 Delivery of luciferin solution (A) compared to luciferin loaded nanocarriers (B; non-ionic surfactant vesicles, NIVs) to luciferase-expressing cells. Mice, infected intravenously with 2 x 10^7 luciferase-expressing *Leishmania donovani* promastigotes, were imaged with luciferin solution (5 mg/ml luciferin in PBS pH 7.4) or luciferin NIVs (30 mM lipid, 5 mg/ml luciferin in PBS pH 7.4). Bioluminescence was observed using IVIS imaging system (PerkinElmer, London UK) and represented as photons emitted per second (C). Treatment with luciferin-NIV delivered significantly more luciferin to the luciferase-expressing parasites at this time point ($p \leq 0.001$).

Drug delivery systems

Incorporation of drugs into a DDS can improve their therapeutic efficacy by directing a drug to the correct site for uptake. The lungs are rich in macrophages and 3% of the cells in the alveolar region are alveolar macrophages and recent studies indicate that lung macrophages self-renew *in situ* and can repopulate locally after tissue damage [35]. Macrophages clear particles from the circulation and using a nanoparticulate DDS will favour macrophage uptake in macrophage-rich tissues and away from urinary excretion [36, 37]. DDS can be produced in different forms to suit a particular clinical condition being treated and/or the route of administration e.g. capsules, vesicular formulations or nanoparticles (NP) and from different constituents e.g. chemically synthesized or natural products. This review will only consider two types of DDS; namely non-ionic surfactant vesicles (NIV), and nanoparticles prepared from natural

polymers, for pulmonary delivery. There are excellent reviews on using types of DDS for pulmonary delivery that may be of interest [37, 38, 39].

Non-ionic surfactant vesicles as a DDS

The properties of NIV depend on their constituents, the relative molar ratio of the constituents and the method used to prepare the NIV. The inclusion of cholesterol into NIV helps stabilise the vesicular membrane and modifying the concentration of cholesterol present in the formulation influences drug loss across the vesicular bilayer. Inclusion of an amphiphile such as dicetyl phosphate, gives the vesicles a net negative surface charge, which helps to keep the vesicle dispersed within a suspension and prevents them clumping [40]. NIV have been used for delivery of a variety of drugs and hydrophilic drugs are entrapped within the aqueous space formed by the vesicle bilayers whereas hydrophobic drugs can be incorporated into the lipid bilayers. NIV can be formed from a single or multiple bilayers depending on the production method. And the size of the vesicles formed can be reduced using different methods post-production e.g. sonication, extrusion under high pressure or homogenisation. In our studies we have used rodent model of visceral leishmaniasis (VL), where animals infected with the protozoan parasite *Leishmania donovani*, allowed us to study drug delivery to the spleen, liver and bone marrow in the same animal [34]. In addition, this model allowed us to investigate local delivery to macrophages as the parasite lives with macrophages within these sites. We used reduction in parasite burdens as well as determining antimony levels within tissues as a measure of drug delivery. This was particularly useful as SSG is a highly water soluble drug and has a short half life. VL is an important neglected tropical disease, which causes 40,000 deaths/year in the Indian subcontinent, would benefit from the production of more effective drug formulations, as there are a limited number of drugs available for treatment [41]. At present there is only one oral drug licenced for treatment of VL i.e. miltefosine, and resistance to miltefosine can easily be induced in the laboratory by culturing the parasite in medium containing increasing amount of miletfosine. Therefore, there is growing concern that the clinical utility of miltefosine may follow the same path as SSG especially as incidences of increased resistance to this drug have already been reported in India [42] and Nepal [43].

In our initial studies we showed that the NIV could be used to entrap the anti-leishmanial drug, sodium stibogluconate (SSG), and that the amount of drug entrapped within vesicles significantly affected their *in vivo* efficacy in different sites. Thus treatment with SSG-NIV at a dose of 44.4 mg Sb^v/kg resulted in a significant reduction in spleen, liver and bone marrow parasite burdens, but only if multiple doses (i.e. 5 doses) were used [44]. Similar treatment with SSG solution could only suppress liver parasites burdens in infected mice. Determination of antimony levels showed that treatment with SSG-NIV resulted in significantly lower blood levels than similar treatment with free SSG solution and significantly higher amounts of antimony were detected in the liver at 6 days post-dosing [36]. Increasing the SSG concentration to prepare SSG-NIV increased the efficacy of the formulation. Thus, single dose treatment with SSG-NIV, prepared using SSG solution at 33 mg Sb^v/ml, significantly suppressed parasite burdens (> 98% compared to controls) in all three sites when animals were treated with a dose of 296 mg Sb^v/kg. In contrast similar treatment with SSG solution only affected hepatic parasite burdens. This SSG-NIV formulation did not require sonication to reduce vesicle size. It was as effective as AmBisome, a liposomal formulation of amphotericin [45], and was highly active against clinical strains of antimony susceptible and antimony resistant *L. donovani* in murine studies [46]. Studies in the dog were carried out to compare the pharmacokinetic and toxicity profile of SSG solution, SSG-NIV, SSG-dextan solution, and a SSG-NIV-dextran formulation. The SSG-NIV-dextran formulation was produced using an additional ultrafiltration step to remove unentrapped SSG, and the dextran was used to balance the osmotic pressure across the vesicle bilayer, which could result in loss of entrapped drug. The mean vesicle size was lower for the SSG-NIV-dextran solution and the entrapment efficiency was seven times higher (mean size: SSG-NIV, 526 nm, SSG-NIV-dextran 253 nm; entrapment efficiency, SSG-NIV, 6%, SSG-NIV-dextran 43%). Treatment of dogs with a single intravenous dose of the four SSG formulations (10 mg Sb^v/kg) showed that the SSG-NIV-dextran formulation gave a significantly highest distribution half-life ($p = 0.01$), longest elimination half-live ($t_{1/2\beta}$) and a significantly higher residence time ($p = 0.02$). There were signs of acute toxicity in dogs treated with this formulation but not the SSG-NIV formulation, but these were short lived and

are probably related to the proportionally higher antimony dose directed to the liver but the formulation. These could be avoided by simply reducing the drug dose. The toxic side effects were those expected for antimony and are unlikely to be related to the DDS as they were absent in dogs treated with the SSG-NIV formulation. Studies using the same formulations in mice showed that the SSG-NIV-dextran formulation was more effective than SSG or SSG-NIV even though it was given at a seventh of the drug dose (33 versus 222 mg Sb^v/kg, [47]). However further development of the SSG-NIV formulation was stopped when antimony resistance developed within endemic parasites became widespread in India, as this may have affected the clinical utility of this formulation. However, this did not stop development of this DDS as we had already demonstrated that NIV could be used to increase the *in vivo* efficacy of other drugs with different physiochemical characteristics [48, 49]. Over the years we have changed the method used to prepare NIV from a solvent based method to a simple 'melt-method' where the vesicular constituents (surfactant, cholesterol, and dicetyl phosphate) were melted at 130°C and then hydrated with drug solution at a temperature of 70°C. We have used homogenisation at different speeds, and for different periods of time, to reduce vesicle size and we have produced vesicle suspensions with different means sizes (100-2000 nm range). We have identified a production method that is suitable for large-scale manufacture, and we have produced litre batches of NIV drug suspensions.

The studies on formulating NIV drug formulations for the intravenous route have helped us develop NIV formulations for administration by the pulmonary route. Using an amphotericin-NIV formulation (AMB-NIV) as our exemplar we have shown that treatment of rats infected with *Aspergillus,* an important human pathogen*,* resulted in a significant reduction in fungal lung burdens ($p < 0.01$). One dose of AMB-NIV was as effective as 5 oral doses of the antifungal drug posaconazole [34]. Treatment with inhaled AMB-NIV resulted in significant higher levels of AMB in the lungs ($p < 0.05$) that similar treatment with AMB solution and significantly lower plasma levels ($p < 0.05$). This formulation was also active against *L. donovani* in a murine model when given by inhalation. Thus treatment with five doses of AMB-NIV resulted in a significant reduction in liver parasite burdens ($p < 0.05$) but failed to affect splenic or

bone marrow burdens compared to controls, the inability to affect parasites in deeper tissues probably reflects poor drug delivery to these sites. Similar studies using mice infected in the footpad with luciferase-expressing *L. major,* a species that causes cutaneous leishmaniasis, showed that NIV did not enhance delivery of luciferin to parasites at this site based on bioluminescence emitted from the footpad after treatment. Therefore, it was not surprising that treatment with inhaled AMB-NIV failed to reduce parasite burdens at this site compared to control values. One of the problems associated with using the murine model is the practical difficulty encountered in treating mice by inhalation. In our studies we have exposed mice to drug formulation by placing them in a Volumetric Spacer, and introducing aerosolised drug formulations produced using a Buxco® nebulisation system into the Spacer. Simple calculation of the drug dose administered using the breathing rate of mice indicates that only a fraction of a dose given is likely to be inhaled by mice. Thus calculation of the best-case scenario indicated that mice would inhale 17% of the drug dose given. It is likely to be much lower, mice given luciferin-NIV by inhalation emitted < 4% of the bioluminescence emitted by mice given the same dose of luciferin-NIV by the intravenous route [34]. Despite this limitation this model can be used for initial screening of inhaled formulation, but ideally a larger rodent model should be used in studies. Other researchers have also determined the feasibility of NIV prepared from a different surfactant for pulmonary delivery of beclomethasone dipropionate (BPD). The amount of drug entrapped within BDP-NIV increased as the drug concentration used to prepare NIV was increased, which is consistent with our findings. The BPD-NIV had a MMAD of 2 µm indicating that the NV would deposit within the lower airways of the lungs. In addition, drug permeation studies indicated that the presence of the non-ionic surfactant increased the ability of the drug to pass through mucin, using *an in* vitro system [50]. Niosomes (or NIV) containing 5-fluorouracil which were prepared using different sorbitan monoesters had a mean size of 3.9-8.1 µm, making some of them too large for inhalation and the smaller ones would be difficult to aerosolise in a droplet size of < 5 µm required for delivery into the lower airways. However, another option would be to produce lyophilised formulations that could be administered as a dry powder using a suitable inhaler. On reaching their deposition

site within the lungs the NIV constituents would be hydrated and vesicles, which would be taken up by the macrophages present [39].

Nanoparticles produced from natural polymers as a DDS

A different type of DDS to NIV are nanoparticles, which can give have high drug loading and are also readily taken up be aveolar macrophages [39]. NP can be produced from biocompatible polymers from natural proteins or sugars. These types of formulations are often produced as a lyophilised product and administered using a dry powder inhaler. This usually requires inclusion of a carrier into the formulation such as lactose, which prevents the NP clumping together. However, by selecting an appropriate inhaler e.g. Turbuhaler® it is possible to remove the requirement for a carrier in the NP formulation [51].

Three natural proteins can be used to produce NP: collagen, albumin or gluten. Production of collagen NPs usually requires the use of solvents and a multi-step method, which would increase manufacture costs. Electrospray methods can be used but these need to be adapted to ensure that particles rather than fibres are produced [52]. Collagen NP with a size of < 1 µm were produced when a salt solution as well as acetic acid at a concentration of < 90% v/v was used. Inclusion of the salt allowed formation of dried NP particle after spraying and increased the conductivity of the particles produced. Scanning electron studies showed that changing the salt solution (i.e sodium chloride vs. calcium chloride), the relative concentration of salt and/or acetic acid allowed NP with mean sizes between 228-900 nm to be formed. Loading collagen NP with theophylline, a drug used in the treatment of chronic obstructive pulmonary disease, resulted in the production of larger particles (size range of 2-3 µm). And factors such as type of nozzle used (single or coaxial), or cross-linking by exposing the formulation to glutaldehyde, influenced NP size; and the amount of cross-linking introduced influenced drug release. No *in vivo* studies were completed in this study but it did show that collagen nanoparticles of a suitable size range for lung delivery could be produced using a single step method.

Albumin NP can be produced using ovalbumin, serum albumin or human albumin. Human albumin is the most appropriate for clinical drug formulations, as it would not induce an immune response [53]. Choi and co workers [54] prepared NP using human serum albumin (HSA) conjugated with doxorubicin and octyl aldehyde. The NP were coated with TRAIL protein (tumour necrosis factor (TNF)-related apoptosis-inducing ligand) to improve drug targeting to cancer cells and had a particle size of 342 nm. The formulation was introduced into the lungs of nude BALB/c bearing lung tumours, caused by implantation of H226 cells. Mice were treated with the NP formulation by the pulmonary route using a microsprayer aerosoliser. Treatment resulted in a significant reduction in tumor burden, based on lung weight and more apoptopic cancer cells were presented in drug treated mice compared to controls. This formulation was more effective than NP prepared using doxorubicin and octyl aldehyde. Data on drug entrapment for this formulation were not given but results from this study indicate that inclusion of a ligand that can bind to cells can improve drug targeting. Two albumin NP formulations (termed Nab) have already been given FDA approval for clinical studies i.e. Nab paclitaxel (Nab-paclitaxel) for the treatment of metastatic breast cancer when used alone [54] or given in combination with carbinoplatin. The overall response rate (42% vs. 23%; $p = 0.022$), and tumour size shrinkage (37v% vs. 20%, $p = 0.006$), was higher in patients with visceral dominant disease treated with Nab-paclitaxel compared to paclitaxel alone. But there was no increase in the mean survival rate for Nab-paclitaxel treated patients compared to paclitaxel treatment alone. Nab-paclitaxel was however given a higher drug dose compared to paclitaxel alone (Nab-paclitaxel, 260 mg/m^2 every 3 weeks, paclitaxel 175 mg/m^2 every three weeks). Drug doses were not matched, as the objective of the study was to examine the efficacy and safety of Nab-paclitaxel versus paclitaxel in patients with poor prognostic factors. The two drugs had a similar toxicity profile but interestingly the NP formulation was more effective in patients ≥65 years old (overall response rates Nab-paclitaxel 27 % vs. 19 % for paclitaxel; progression free survival 5.6 vs. 3.5 months), which is important factor as elderly patients are now more common within populations [56, 57]. A more effective drug formulation for this cohort of patients would be beneficial.

NP can be made from polysaccharides such as chitosan, hyaluronate, cellulose, carrageenans, alginate or starch. Chitostan is a cationic polysaccharide usually produced from chitin by deaceylation. Chitin has a pK_a of 6.2-6.8, and within an acidic environment (e.g. within tumour cells); chitosan can remain protonated and will swell. This would favour quick release of drug at the deposition site of drug-loaded NP. Chitostan NP loaded with the anticancer drug methylglyoxal, were small (50-100 nm), and had a net positive charge (+24 mV), and released their drug load within 10-12 hours [58]. The small size of these NP may indicate that the NP have the potential to be exhaled once they are released into the airways from their aerosol droplet. Topotecan-loaded NP produced from chitosan were much larger, with a size of 642 nm and a positive surface charge of 35 mV. These NP had a drug entrapment efficiency of 100% when a topetecan: chitosan ratio of 1:20 was used. In contrast a poly(D,1-lactide-co-glycide or PGLA) and topetecan-loaded NP composite, where the NP were coated in PGLA resulted in a formulation with a lower entrapment efficiency (28%). This is probably due to drug loss when PGLA cross-linked to chitosan. The composite particles had a mean size of 2.1 µm and the surface charge dropped to -6.99 mV. A net negative charge may be beneficial as a positive surface charged has been associated with cytotoxicity for liposomes [59]. Drug release from the composite particles was much slower so that only 24% of the entrapped drug was released during the first 24 hours, making these NP suitable for sustained drug release at their uptake site.

Hyaluronate can also be used to improve targeting to cancer cells as it binds to CD44 and CD168, markers upregulated on cancer cells [60]. NP loaded with paclitaxel and baicalcein, had a hydrophilic shell of hyaluronate and a hydrophobic core that contained the drugs. The NP had a mean size of 92 nm and a zeta potential of + 3 mV and the nanoparticulate formulation was more toxic to A549 cells then paclitaxel/ baicalcein solution alone ($p < 0.05$). Studies in a murine tumour model, where Kumming mice were injected with paclitaxel resistant A549 cells, showed that a single intravenous treatment dose significantly inhibited tumor growth ($p < 0.05$). In contrast similar treatment with the individual drugs had no significant effect on tumour size compared to controls. Only treatment of mice with paclitaxel or baicalcein solution

resulted in a noticeable reduction in weight compared to controls, indicating that the NP drug formulation had a lower toxicity to mice.

Ethyl cellulose was used to produce NP by preparing using an oil in water emulsion solvent technique [60]. The resulting formulation was exposed to spray freeze-drying or spray drying, to produce nanocomposite microcarriers from the NP, which would break up to release the NP from the composite in an aqueous environment. The initial NP had a mean size of 111 nm before spray drying or spray freeze-drying. After spraying the nanocomposite microcarriers had a mean volume size of 7.2 µm using spray drying and 12.3 µm for spray freeze-drying. Spray freeze-drying gave particles that had a much larger surface area (77.6 vs. 2.4 m^3/g) but the MMAD (2.4 µm vs. 3.1 µm) and GSD (3.1 vs. 2.9) were very similar for the two spraying methods. Reconstitution of the nanocomposite microcarriers showed that spray freeze-drying gave a better formulation as it resulted in release of NP with a more uniform size [61]. The results indicate that this method could produce a NP based formulation that was suitable for pulmonary delivery, that could be administered as an inhalable dry-powder. Ethyl cellulose or a mixture of ethyl and methyl cellulose were used to prepare NP that contained an extract from *G. mangostana* Linn, a tropical fruit from Southeast Asia [61]. The NP had a similar mean size of 253 and 250 nm respectively and a similar drug entrapment efficiencies (86 vs. 88%). NP prepared from ethyl cellulose had a more negative ζ-potential (-31 vs. -12 mV respectively). Both formulations were cytotoxic to HeLa cells, with NP prepared using ethyl cellulose being more cytotoxic (IC_{50} values 16.7 vs. 7.4 µg/ml). The corresponding extract-free NP had no toxicity against the cells at the doses used.

NP were formed from alginate using poly(lactic-co-glycolic acid) [PLGA] and chitostan or poly(vinyl) alcohol [PVA]. Alginate/PLGA/chitostan NP had an entrapment efficiency of 71% (502H PGLA) or 80% (756 PGLA) whereas NP containing PVA instead of chitostan had an entrapment efficiency of 79% (502 H PGLA) or 61% (756 PGLA). NP that did not contain alginate had very poor drug entrapment (<10%), indicating that inclusion of alginate gave better drug loading. Tobramycin loading of these NP gave PV NP with a mean size of 300 nm whereas PGLA 756 NP were larger (400-500 nm).

Tobramycin loaded NP formed from alginate/PGLA/chitostan had a higher surface charge (20 to 40 mV) compared to NP formed from alginate/PGLA/PVA (approximately -5 mV). Both types of NP released tobramycin slowly so that drug was still present in the medium at day 40 in *in vitro* drug release studies. The two types of NP were loaded with rhodamine and produced as a dry powder using a spray drying method. This method produced larger structures that were termed 'nano-embedded microparticles' (NEM). The two types of NEM formulations had a similar MMAD (alginate/502 H PGLA/chitostan, 3.7; alginate/502 H PGLA/PVA, 3.7), with alginate/502 H PGLA/chitostan NEM having a higher FPF value (38 vs. 52% respectively). Rats were treated by the pulmonary route with the different NEM formulation using a breath-activated, reusable DPI to determine where they deposited in the lungs. Rhodamine alginate/PGLA/chitostan NEM deposited rhodamine in the trachea, bronchia and bronchioles whereas alginate/PGLA/PVA NEM deposited rhodamine in the alveolar ducts and not the upper airways [62].

There are a number of studies on using starch to prepare DDS, as it is one of the main dietary carbohydrates, and is therefore safe to use in humans. Starch was used to prepare funtionalized graphene nanosheets and loaded with hydroxycamptothecine (HCPT). 12 µg or HCPT could be loaded in to 150 µg of starch-graphene complex. At the doses used, co-incubation of SW-620 cells had no cytotoxic effect against cells whereas treatment with HCPT-starch-graphene or HCPT were toxic to cells. The drug solution was more effective but this could reflect the slow release of the drug from the graphene composite. Obviously for pulmonary delivery this type of complex would have to be manufactured into a suitable size and particle size for inhalation, and careful selection of the type of graphene to avoid toxicity is required [63].

Conclusions

Both NIV and NP produced from natural products can be used to deliver a variety of drugs by different routes including inhalation. In both cases a switch from batch manufacture to continuous manufacturing processes would facilitate large-scale production [64] and studies have shown that spray drying, which could be incorporated into such a method, is feasible for some formulations. Lactose is often

used as a carrier to protect drugs against the harsh environmental conditions present during spray drying, and it often mixed with drug formulations to improve the flowability of powders administered using dry powder inhalers [65]. Therefore it is important to consider what type of device is going to be used for a drug formulation early on in its development to ensure that the appropriate type of formulation for the intended delivery device is developed. Three-dimension printing is an area that is actively being explored for production of production of novel drug formulations but achieving the small size required for production of nanoparticles is challenging, but not impossible. Nanoimprint lithography has been used to produce shape-specific solid NP with sizes of 50-400 nm [66]. And Cylindrical nanoparticles with a diameter of 240 or 125 nm have been produced using poly (acrylic acid), which could be used for aqueous or organic solvent-based imprint solutions [66]. Adapting 3-D printing methods to prepare NP from natural proteins or polysaccharides may be challenging, but alginate is already been used in preparing 3-D printed hydrogels, as it is a viscous non-toxic material that cross-linked in the presence of some divalent cations [68]. One major hurdle for translating a laboratory formulation into a clinical product is successful completion of preclinical toxicity testing. However it is difficult predict whether a formulation will indeed be safe even if natural instead of synthetic, surfactants are used in the DDS as the entrapped therapeutic may be cytotoxic. However using a surfactant that is biocompatible with humans should reduce the inherent toxicity of the DDS. At present healthcare providers are struggling to treat the high number of patients that use public health services, whilst meeting the constraints of their budget. Production of drug formulations or reformulation of existing drug for a non-invasive administration route could be a beneficial as it could reduce treatment costs if patients self-mediate rather than rely on in-patient service for drugs currently given by the parenteral route. Production of an effective drug formulation that can be given by inhalation may be facilitated by co-development of a nebuliser/drug formulation combination, to ensure that the formulation is deposited in the correct area of the respiratory tract for the particular disease indication. Technology to produce aerosols of drug formulations has changed to try and controls some of the ‘drug-free’ factors that can impact on therapeutic outcome, such as patient profile (adult versus pediatric use), device used, as well as correct use of the

481 inhalation device by the patient [16, 51, 56]. Therefore testing what impact using
482 different nebulisers/inhalers has on pulmonary delivery should be an important
483 consideration in the development of any inhaled drug formulation.

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