PULMONARY DRUG DELIVERY AS A VITAL ROUTE FOR DELIVERING NANOPARTICLES – A REVIEW

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ABSTRACT

The nanotechnology has reignited interest in the lungs as a main route of drug delivery for both systemic and local treatments. The large alveolar surface area of lung coupled with the thin epithelial barrier and extensive vascularization might enhance drug transport and uptake. Such an avenue for drug delivery would greatly improve the treatment of pulmonary diseases such as asthma, chronic obstructive pulmonary diseases, lung cancer, tuberculosis and cystic fibrosis etc. The alteration of particle characteristics including size, surface chemistry, surface charge, and surface area, allows for the possibility of synthesizing particles specific for targeting to various cellular populations and organs including systems such as the respiratory tract. Pulmonary drug delivery is an area extensively investigated through the use of nanoparticles (particles that have a mean diameter of 300 nm or less). Polymeric nanoparticulate formulations have many advantages over traditional dosage forms which include enhanced dissolution properties and vast potential for intracellular drug delivery. It is analysed that in the near future pulmonary diseases may be treated effectively and efficiently using inhalable therapeutic agents loaded nanoparticles. Such an approach might minimize drug related systemic side effects. After identification of the various modes of interaction, it will be possible to control and minimize such toxicological effects allowing for nanoparticles to be safely utilized as therapeutic agents for site-specific drug delivery.

Keywords: Nanoparticles, pulmonary drug delivery, aerosol, alveoli, bronchioles, inhalation,
site-specific targeting.

INTRODUCTION
The pulmonary route has recently gained importance as a suitable target for drug delivery. The large alveolar surface area, the thin epithelial barrier and extensive vascularization have the tendency to enhance drug transport and uptake. Pulmonary drug delivery is a non-invasive, non-systemic delivery approach to directly target disorders of lung (such as asthma, chronic obstructive pulmonary diseases (COPD), emphysema, cystic fibrosis, lung cancer, tuberculosis, pulmonary hypertension and diabetes etc) for both systemic and local drugs. It offers several advantages\(^1\) over oral, intranasal and transdermal delivery systems such as large absorptive surface area with extensive vasculature, easily permeable membrane, immense capacity for solute exchange due to thinness of the alveolar epithelium, low extracellular and intracellular enzymatic activity, rapid and predictable onset of action with minimum side effects, reduced costs due to need of low dose, avoid first pass metabolism and improved patient compliance\(^2\).

![Figure 1: Different regions of the human respiratory tract](image)

The human lung (figure-1) consists of two functional parts, the airways (trachea, bronchi and bronchioles) and the alveoli (gas exchange areas). It contains about 2300 km of airways and 500 million alveoli. The surface area of the human lungs is estimated to be approximately 75-140 m\(^2\) in adults\(^3\). The pseudostratified epithelia that constitute the barrier to absorption into the blood-stream are markedly different in airways and alveoli of the lungs. The airways are...
composed of a gradually thinning columnar epithelium, with the bronchial epithelium of 3-5 mm and bronchiolar epithelium of 0.5 – 1 mm in thickness. In the tracheo bronchial region the epithelium is protected by a mucus layer. Any particle deposited in this area is transported away from the lung by mucociliary clearance or diffuse through the thick mucus to reach the epithelium cells. In contrast, the alveoli have a thin, single cell layer. The distance from the air in the alveolar lumen to the capillary blood flow is less than 400 nm. The large surface area of the alveoli and the intimate air-blood contact in this region make the alveoli less protected against inhaled substances, such as nanoparticles as compared to the airways\(^4\). The pulmonary drug delivery system is based on the principle of aerosolization. Aerosols containing a uniformly sized particle matrix, which carry a drug or a single drug-containing matrix, may provide uniform dose delivery and drug release kinetics. Pulmonary lung targeting finds applications in drug delivery\(^5\) to (1) lung itself (ie. local delivery) (2) other body organs after dissolution and subsequent absorption into blood circulation following transfer across alveolar membranes (ie. Systemic delivery) (3) metabolism by alveolar macrophages\(^6\). To generate aerosols, containing nanometer-sized dry particles from suspensions, there are two methodologies (airjet atomization and electrohydrodynamic atomization). In air jet atomization, atomizer is used to aerosolize suspensions and the generation of nanometer sized dry particles results from the evaporation of drops. Electrohydrodynamic atomization (EHDA) allows control or tuning of the droplet size, through control of conductivity of the liquid\(^7\). Various advantages of aerosol administration are

(i) Rapid drug absorption into the systemic circulation
(ii) Higher bioavailability than other non-invasive route of administration because of delivery of peptides and proteins as compared to oral administration and for many small molecules where first-pass metabolism limit oral bioavailability.

Inhalation devices for pulmonary drug delivery are of three types which as follows

A. Meter dose inhalers
B. Dry powder inhalers
C. Nebulizers

**A. Meter dose inhalers:** A metered - dose inhaler (MDI) (Figure-2) is a complex system designed to provide a fine mist of medicament, generally with an aerodynamic particle size of
less than 5 microns, for inhalation directly to the airways for the treatment of respiratory diseases such as asthma and COPD\(^8\).

**Figure 2: Meter dose inhalers**

**B. Dry powder inhalers:** Dry powder inhalers (DPI) (Figure-3) are frequently highly soluble and quickly dissolve in the fluid layer lining the surface of the deep lung before passing through the thin cytoplasm of the type I alveolar cells, the interstitial space and capillary endothelium. The main advantages of dry powder systems include product and formulation stability, the potential for delivering a low or high mass of drug per puff, low susceptibility to microbial growth, and applicability to both soluble and insoluble drugs. Current challenges facing the development of these systems for macromolecules include moisture control, efficient powder manufacturing, reproducible powder filling, unit dose packaging and development of efficient reliable aerosol dispersion and delivery devices\(^8,9\). There are two types of devices

**Figure 3: Dry powder Inhalers**

i. **Unit-Dose:** Devices Single-dose powder inhalers are devices in which a powder contained capsule is placed in a holder. The capsule is made to open within the device and the powder is inhaled.

ii. **Multi-dose Devices:** Multi-dose device uses a circular disk that contains either four or eight powder doses on a single disk. The doses are stored in separate aluminum blister reservoirs until just before inspiration.
C. **Nebulizers**: There are two types of nebulizer systems, the ultrasonic and the air jet. In ultrasonic nebulizers, ultrasound waves are generated in an ultrasonic nebulizer chamber by a ceramic piezoelectric crystal that vibrates when electrically excited. These set up high energy waves in the solution, within the device chamber, of a precise frequency that generates an aerosol cloud at the solution surface. The aerosol produced by an air jet nebulizer is generated when compressed air is forced through an orifice; an area of low pressure is formed where the air jet exists. Nebulizers (Figure 4) are particularly useful for the treatment of hospitalized or non-ambulatory patients [8].

![Diagram of Air Jet Nebulizer]

**Figure 4: Air jet nebulizer**

Many efforts have been focused for specific delivery of drugs to the target tissues by the development of nanoparticles, solid lipid nanoparticles, liposomes, nanoemulsions, or dendrimers. Nanoparticles are particulate dispersions with a size in the range of 1-1000 nm and the drug is dissolved, entrapped, encapsulated or attached to nanoparticle matrix. In drug delivery system, the roles of polymeric nanoparticles are to carry the drug molecules, to protect drugs from degradation, and to control drug release. Polymeric nanoparticles used therapeutically are composed of biodegradable or biocompatible materials, such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), alginic acid, gelatin and chitosan [10,11]. Nanoparticles as a drug delivery system [10] have several advantages:

a) An achievement of enhanced solubility of the drug than its own aqueous solubility

b) Site-specific targeting can be carried out by attaching targeting ligands to surface of particles or use of magnetic guidance.
c) Sustain release of the drug at the time of transportation and at the site of localization to obtain increase in drug therapeutic efficacy and reduction in side effects by changing distribution of the drug in organ and clearance of the drug.

d) Increased potential of drug internalization by cells.

e) Particles degradation and controlled release characteristics can be easily modulated by the choice of matrix constituents.

f) Drug loading is high and drugs can be incorporated into the systems without any chemical reaction which helps in preserving the drug activity.

g) Improved patient compliance.

CAPABILITY OF NANOPARTICLES TO TARGET LUNG TISSUES

(A) Mechanism of deposition of nanoparticles in the respiratory tract: Size particularly is an important determinant of whether or not nanoparticles will be efficiently deposited deep into the lungs or if they will simply be exhaled. The sizes of particles which are used for inhalation therapy are usually expressed in terms of the mass median aerodynamic diameter (MMAD)\(^\text{[12]}\). The aerodynamic diameter is usually defined as the diameter of a sphere of unit density, which reaches the same velocity in the air stream as a non-spherical particle of arbitrary density. This diameter defines the mechanism of particle deposition in the respiratory system\(^\text{[13]}\). In general, aerosol particle size is considered to be the mass median aerodynamic diameter (MMAD). The MMAD is used to explain the particle size distribution of any aerosol statistically based on the weight and size of the particles. Thus, a group of very dense particles will exhibit a larger MMAD than that of a group of less dense particles, despite an identical geometric size\(^\text{[14]}\). The criterion for successful deposition requires that the particles must be small enough to avoid deposition by impaction in the upper respiratory tract and enable them to pass through the mouth, larynx, pharynx, and lower airways while simultaneously being large enough (or having sufficient inertia) to avoid exhalation\(^\text{[5]}\). Consequently, particle size and density reflected in the MMAD of a particle are important characteristics for lung delivery. The nanoparticle diameter ranging from 30-50 nm and 1-3 \(\mu\text{m}\) have found to exhibit high deposition in pulmonary region\(^\text{[15]}\). Different studies have shown that particles containing diameters ranging from 100–500 nm might be successfully deposited into various regions within the respiratory, tract when they are incorporated into suitable vehicles such as aerosols or dry powders\(^\text{[10,13]}\).

Particle deposition occurs via on of the following principal mechanisms(Figure-5):
1) **Impaction**: Each time the airflow changes due to a bifurcation in the airways, the suspended particles tend to travel along their original path due to inertia and may impact on an airway surface. This mechanism is highly dependent on aerodynamic diameter, since the stopping distance for very small particles is quite low. Impaction occurs mostly in the case of larger particles that are very close to airway walls, near the first airway bifurcations. Therefore, deposition by impaction is greatest in the bronchial region. Impaction accounts for the majority of particle deposition on a mass basis.\[16\]

2) **Sedimentation**: Sedimentation is the settling out of particles in the smaller airways of the bronchioles and alveoli, where the air flow is low and airway dimensions are small. The rate of sedimentation is dependent on the terminal settling velocity of the particles, so sedimentation plays a greater role in the deposition of particles with larger aerodynamic diameters. Hygroscopic particles may grow in size as they pass through the warm, humid air passages, thus increasing the probability of deposition by sedimentation.\[16\]

3) **Interception**: Interception occurs when a particle contacts an airway surface due to its physical size or shape. Unlike impaction, particles that are deposited by interception do not deviate from their air streamlines. Interception is most likely to occur in small airways or when the air streamline is close to an airway wall. Interception is most essential for fibers, which easily contact airway surfaces due to their length. Furthermore, fibers have small aerodynamic diameters relative to their size, so they can often reach the smallest airways.\[16\]

4) **Diffusion**: Diffusion is the primary mechanism of deposition for particles less than 0.5 microns in diameter and is governed by geometric rather than aerodynamic size. Diffusion is the net transport of particles from a region of high concentration to a region of lower concentration due to Brownian motion. Brownian motion is the random wiggling motion of a particle due to the constant bombardment of air molecules. Breath holding is essentially for deposition of nanometer particles where the mechanism of deposition is predominantly diffusion.\[17\]

5) **Electrostatic precipitation**: Electrification normally occurs in aerosol generating processes. The unipolar charge carried by the particle may have a significant effect on the deposition efficiency of particles in the lungs during breathing. The deposition of the particles in the lungs shows a relatively small fractional deposition in the lungs under same conditions when condensation aerosols are used. Since, the condensation, aerosols are free of electric...
charges, the difference in fractional deposition may be caused by the additional deposition from electrostatic forces. This type of effect is quite significant for submicron and micron particles carrying unipolar charge of magnitude of hundred electrons. The observed changes are caused primarily by electrostatic precipitation resulting from the image force between the particle and the wall\textsuperscript{[18]}.

![Figure 5: Deposition mechanisms of particles](image)

The area in the respiratory tract for where the deposition of nanoparticles will occur primarily depends on their size\textsuperscript{[3]}. 1 nm particles deposition will primarily occur within the upper airways including the nose, pharynx, and larynx\textsuperscript{[3]}. Optimal deposition into the tracheal and bronchi regions requires the use of 5 nm particles and 20 nm particles are optimal for deposition into the deeper alveolar regions of the lungs\textsuperscript{[19]}. The inertial impaction occurs during the passage through the oropharynx and large conducting airways if the particles possess a mass median aerodynamic diameter (MMAD) more than 5\(\mu\)m. When the MMAD of particles ranges from 1 to 5 \(\mu\)m, they are subjected to sedimentation by gravitational force that occurs in smaller airways and respiratory bronchioles. Sedimentation is influenced by breath holding. Particles with a MMAD of less than or equal to 0.5 \(\mu\)m, they are deposited significantly by diffusion, based on the Brownian motion\textsuperscript{[4]}.

(B) Interaction of nanoparticles with lung epithelia and macrophages

Depending upon the location of deposition, the nanoparticles will interact with specific cell population within the lungs (Figure – 6). Ciliated epithelial cells as well as type I and II pneumocytes are the primary cells within the deep respiratory tract that interact with
nanoparticles\textsuperscript{19}. However the mechanisms of interaction are not well known and very little exists in the literature.

![Diagram of nanoparticle interaction with lung epithelia and macrophages.](image)

**Figure 6: Interaction of nanoparticles with lung epithelia and macrophages**

Receptor-mediated endocytosis is the mechanism most likely responsible for the intracellular uptake of the nanoparticles whereby opsonins (such as proteins, glycoproteins, and glycolipids) precipitate onto the particle surface forming a complex recognizable by receptors of macrophages which then may bind to the complex with their cell surface allowing for particle uptake through pseudopod extensions. Phagosomes then fuse with lysosomes (containing acid hydrolyases) which have the ability to degrade the drug delivery vehicle. During this process however, the drug itself as well as the particle may be destroyed by the action of enzymes and thus the nanoparticles must first emigrate the lysosomes in order to retain activity. Nanoparticle delivery to the alveolar region allows drug targeting to the alveolar macrophage population and has important magnitude for treating diseases which involve or are caused by these immune cells. Furthermore, nanoparticles have potential to be used for delivery of antigens and DNA and may be important for vaccine delivery through the respiratory route\textsuperscript{20}.

Macrophages are central in defending the lungs against the assaults of particles and pathogens in inspired air. Particles are not only ingested but undergo gradual dissolution within the phagolysosomes of macrophages. The phagocytic and microbicidal potential of macrophages is one of the major reasons to keep lungs remain clean and sterile. Macrophages...
may also prevent allergy by ingesting and catabolizing inhaled foreign particles. During lung infections macrophages may preserve and present antigens to lymphocytes and act cooperatively with other components of the immune system to enhance the immune response. Lung macrophages recognize and destroy neoplastic cells, thus preventing the development of cancer\(^\text{[21]}\). Macrophages can secrete such diverse substances as lysosomal enzymes, interferon, components of complement, angiogenesis factor, plasminogen activator, cyclic nucleotides, leukotrienes, prostaglandins, inflammatory cytokines, and granulopoietins. Diverse agents such as viruses, silica, immunosuppressives, ethanol intoxication, cigarette smoke, air pollution, hypoxia, and hyperoxia can depress the ability of pulmonary macrophages to protect their host. There are also situations in which pulmonary macrophages not only fail but are themselves implicated in the pathogenesis of pulmonary diseases. For example, the ingestion of particles (e.g., cigarette smoke), microbes, or endotoxin causes the release of lysosomal enzymes and oxygen radicals into the macrophage cytoplasm or the external environment. These substances may damage surrounding cells or other macrophages; then dead or dying macrophages release substances that can attract fibroblasts and elicit fibrogenic responses. This extracellular release of proteases and oxygen radicals can also alter the extracellular matrix or the activity of a variety of enzymes, then macrophages may be centrally involved in the development of lung disease\(^\text{[21]}\).

(c) **Interaction of nanoparticles with lung surfactant:** Once nanoparticles are deposited onto the lining of the respiratory tract, they first contact the mucous layer within the airways or the surfactant – lining fluid layer within the alveolar region (Figure-7). Airway mucous (about 5 µm in depth) is a complex aqueous secretion of airways, comprising electrolytes, proteins, glycoproteins (mucins) and debris of cells. The components vary much depending on environmental and disease states. The surfactant lining layer (10-20 nm in thickness) that covers the alveolar surface is composed of 90% in weight of phospholipids and 10% in weight of specific proteins\(^\text{[22]}\). Both airways and alveolar surface liquids are coated with at least a monolayer of highly surface active lung surfactant, which are primarily water insoluble long-chain phospholipids. They form liquid crystals but not micelles in aqueous media to maintain the functions of the lungs such as facilitation of gas exchange and prevention of alveoli collapse by reducing the lung air interface surface tension\(^\text{[23]}\).
Figure 7: Synthesis and secretion of surfactant which coats the alveolar surface of the air

Water interphase.

An analysis of the interaction between pulmonary surfactant and nanoparticles is of utmost importance as dire consequences would result if such drug delivery systems destabilize the surfactant film coating the alveoli\textsuperscript{[24]}. Accordingly many surfactant film studies are performed using a Langmuir Blodgett trough which allows one to mimic the physiological situation present within the respiratory tract\textsuperscript{[18]}. A recent study by Stuart et al. investigated the interaction of different nanoparticles with the lung surfactant film\textsuperscript{[24]}. Accordingly, the interaction between nanoparticles and dipalmitoylphosphatidylcholine (DPPC) a major component of native pulmonary surfactant was investigated\textsuperscript{[18]}. A DPPC lipid monolayer was used to simulate the surfactant layer of the respiratory tract\textsuperscript{[18,24]}. The aim of this study was to investigate if the deposition of nanoparticles in the alveolar region will compromise the integrity of the surfactant film. Such interactions might cause dosage form related incompatibilities and are important for the preclinical evaluation of the feasibility of nanoparticle delivery of nanomedical devices. The incorporation of the particles into the lipid film was size dependent and had a measurable impact on the surface tension of the lipid layer. However, the study also showed that nanoparticles do not significantly destabilize the surfactant film. The method outlined in this study might be a suitable test to set limits for nanoparticle deposition and to evaluate dosage form related nanotoxicological properties of inhalable nanoscaled drug delivery systems. In another study, nanoparticles comprised of D-\textsuperscript{\textcopyright}-Tocopheryl polyethylene glycol 1000 succinate (TPGS) as well as other biodegradable substances as carrier matrix were investigated. TPGS is also known to stabilize pulmonary surfactant thereby making its use in pulmonary drug delivery more attractive. The study also
concluded that the TPGS coated nanoparticles do not destabilize the model surfactant film and have great potential for pulmonary drug delivery\textsuperscript{[13]}.

(d) **Retention and clearance mechanisms of nanoparticles deposited within the respiratory tract:** The nanoparticles must first overcome the clearance and defense mechanisms associated with the pulmonary route for effective deposition. The particles must have the ability to escape the mucociliary clearance within the upper bronchi-tracheal region in order to reach the epithelial cells\textsuperscript{[10]}. The primary function of the mucociliary clearance is to trap inhaled dust particles removing them from the respiratory tract and involves ciliated epithelial cells located within the upper airway (bronchi and tracheal regions). The cells cover between 30–65% of the airway epithelium and each of the epithelial cells bear approximately 200 cilia on their surface, greatly enhancing particle elimination\textsuperscript{[3]}.

These ciliated epithelial cells are surrounded by periciliary fluid which includes a mucous Layer\textsuperscript{[3]} and this fluid enhances the mucociliary clearance by effectively trapping particles. The mucus is pushed towards the glottis by the mucociliary clearance and from here the particles will either be removed via excretion through the mouth or through the gastrointestinal tract. If particles are able to enter the lower airways such as alveoli, then the particles will be cleared through the action of alveolar macrophages. They reside within the surfactant monolayer 3) and nanoparticles are able to interact with them as mentioned earlier\textsuperscript{[3]}. Upon activation the alveolar macrophages release immunological response mediators such as cytokines and chemokines\textsuperscript{[25]} which ultimately allow for phagocytosis and particle elimination. The subsequent pulmonary inflammation caused by such mediators is however disadvantageous as a result of the adverse health effects and toxicity effects observed with the use of ultra fine particles. The inflammation can lead to oxidative stress due to depletion of alveolar anti-oxidants. Pulmonary surfactant also plays a vital role in protecting and eliminating inhaled particles and experiments have shown that the surfactant plays a role in the particle clearance upon expiration. During expiration, a pressure gradient is established which facilitates particle elimination where the high surface tension within the trachea and bronchi (in comparison to the alveoli) forces particles towards the surfactant, facilitating their elimination. Alveolar clearance is instigated as the surfactant film promotes the deposition of these particles within the aqueous region where the resident macrophages await. Once deposited within the lung lining fluid, there are separate biokinetics for lung absorption and non-absorptive clearances. The kinetics of dissolution of inhaled particulates
determines whether the inhaled nanomaterials will dissolve in the epithelial lining fluid for lung absorption or whether such nanomaterials will undergo non-absorptive clearances\[^{26}\]. The kinetics of diffusion in the alveoli is much faster than that in the small airways, mainly because lung absorption mostly occurs from the air-side surface of the alveoli to the pulmonary capillaries. The alveoli has thin monolayer (0.1 – 0.4µm) composed of extremely broad and thin type I cells and small compact type II cells, and a large surface area (more than 100 m\(^2\)), only a small portion of inhaled nanoparticles is absorbed from the tracheobronchial airways which have a much thicker layer of column- shaped epithelial cells (10-60 µm) and lower surface area (1-2 m\(^2\)). This is supported by Fick’s law. Low molecular weight hydrophilic molecules can be absorbed by the active transport via specific transporters or by passing through the tight junctions. The kinetics of active absorption should depend upon the lung regional expression and functionality of receptors or transporters\[^{27}\].

Inhaled nanomaterials that are insoluble in mucus and lining fluid are not able to be rapidly absorbed and may undergo physical translocation. This is different depending on the lung region in which the nanoparticles have been deposited. Immersion of the inhaled, slowly dissolving or insoluble nanomaterials in the fluid lining the lungs may enable them to be closely associated with epithelial cells and cells of the host-defence system for particle-cell interaction. Subsequently, several post-defense mechanisms, including the mucociliary escalator transport, phagocytosis by macrophages and endocytosis are involved in the removal of deposited nanoparticles and to maintain the lung mucosal surfaces\[^{28}\].

The mucociliary escalator dominates clearance of nanoparticles from the upper airways. Nanoparticles that consist of slowly dissolving or insoluble materials in the airway mucus will be partly moved by action of the ciliated epithelial cells pushing the mucus along with the nanoparticles that deposited on the airway wall to the larynx, where they are swallowed to the gastro-intestinal tract or excreted through the mouth. The deposited nanoparticles may also be removed by coughing within 1-2 days\[^{4}\].

Clearance of the slowly dissolving and insoluble nanoparticles from the alveoli is predominantly by macrophage phagocytosis and endocytosis. The airside surface of each of the 500 million alveoli in the human lung is routinely monitored by 12-14 alveolar macrophages in the lung lining fluid. The uptake of deposited particles by alveolar macrophages depends upon the particle size and composition of coating material. Particles of
1-3 µm in diameter are far better taken up than those of 6 µm by macrophages, which have cell diameters about 15-22 µm. Size of particles less than 0.26 µm can escape from the phagocytosis by macrophages[29]. Due to the small size, the chance of nanoparticles undergoing phagocytosis in the alveoli is much lower than micron-sized particles. The remaining nanoparticles will interact with the non-phagocytic cells of the epithelium and the endocytic events are regulated by clathrin-coated pits and caveolae, as well as scavenger receptors. It has been suggested that caveolae and coated pits preferentially transport small and large particles, respectively, but this need to be further verified in vivo. Caveolae are indentations of the plasma membrane lined with caveolin-1, and are abundantly expressed on lung capillaries and type I alveolar cells. Macromolecules or particles of several nanometers in radii may be transported within caveolae from lung to blood[30].

PROCESSING METHODS OF NANOPARTICLE FOR PULMONARY DRUG FORMULATIONS

(1) Emulsion-Solvent Evaporation: In this method, the polymer is first dissolved in a water-immiscible, volatile, organic solvent such as chloroform, dichloromethane, or ethyl acetate. The drug is added to this polymer solution and the mixture is emulsified into an outer water phase containing an emulsifier, such as poly(vinyl alcohol) (PVA), gelatin, polysorbate 80, or polaxamer-188 to yield an o/w emulsion. To harden the nanoemulsion droplets into solid nanoparticles, the organic solvent is evaporated or extracted from the system after it diffuses into the external aqueous phase. Emulsification is facilitated by high-speed homogenization or sonication. For the removal of solvent, the stirring process may be continued for several hours at One of the challenges encountered in this method is the poor entrapment and burst release effect of moderately–water-soluble and hydrophilic drugs[31].

(2) Emulsification Solvent Diffusion Method: In this technique, the solvent and water are mutually saturated at room temperature before use to ensure the initial thermodynamic equilibrium of both liquids. Later, the organic solvent containing the dissolved polymer and the drug is emulsified in an aqueous surfactant solution (usually with PVA as a stabilizing agent) by using a high-speed homogenizer. Water is subsequently added under constant stirring to the o/w emulsion system, thus causing phase transformation and outward diffusion of the solvent from the internal phase, leading to the nanoprecipitation of the polymer and the formation of colloidal nanoparticles. Finally, the solvent can be eliminated by vacuum steam distillation or evaporation[32].
(3) Emulsion Polymerization: This method has been used to prepare poly (alkyl cyanoacrylate) nanoparticles with an approximate diameter of 200 nm. The alkyl cyanoacrylate monomer is dispersed in an aqueous acidic medium containing stabilizers such as dextrans and poloxamers. Surfactants such as polysorbates can be used as well. The low pH favors the formation of stable and high molecular mass nanoparticles. Under vigorous mechanical stirring, polymerization follows the anionic mechanism since it is initiated usually by nucleophilic initiators such as OH\(^-\), CH\(_3\)O\(^-\), and CH\(_3\)COO\(^-\) and proceeds at ambient temperature. The nonpolar ends within the interior of the surfactant micelles help solubilize the monomer. In the presence of water-soluble initiators, chain growth commences at the hydrophilic surface of the micelle. When the monomer in the interior of the micelle gets depleted, more monomer droplets from the exterior aqueous phase enter inside; thus, the polymerization reaction proceeds inward and continues until it is terminated by the free radicals. The drug can be solubilized in the polymerization medium either before the monomer is added or later when the reaction has ended. Finally, the nanoparticulate suspension is purified either by ultracentrifugation or by redispersing the nanoparticles in an isotonic medium. The various factors affecting the formation of particles, their size, and molecular mass include monomer concentration, stirring speed, surfactant/stabilizer type and concentration, and the pH of the polymerization medium\(^{33}\).

(4) Supercritical Fluid Technology: This technology is advantageous in that the use of an organic solvent/surfactant can be avoided or minimized, thus producing nanoparticles that are free from toxic impurities. Carbon dioxide is nontoxic, nonflammable, and environmentally acceptable, and supercritical CO\(_2\) can be easily obtained by pressurizing and heating the CO\(_2\) system to a minimum of 73.8 bars and 31.05\(^\circ\)C, respectively.

In the supercritical antisolvent method, both the drug and the polymer are dissolved in a suitable organic solvent and are atomized through a nozzle into supercritical CO\(_2\). The dispersed organic solvent phase and the antisolvent CO\(_2\) phase diffuse into each other and since CO\(_2\) is miscible only with the solvent, the solvent gets extracted causing the supercritical fluid–insoluble solid to precipitate as nanoparticles. The rates of two-way mass transfer are much faster than those for conventional organic antisolvents. When the density of CO\(_2\) decreases, the atomization of the spray is intensified, resulting in faster mass transfer rates associated with high surface area of the associated droplets, thus rapid nucleation and
smaller particle sizes. The dry, micronized powder is then collected following the depressurization of CO₂[^34].

In the rapid expansion of supercritical solutions technique, the solute is dissolved in supercritical CO₂ and this solution is atomized through a nozzle into a collection chamber at atmospheric conditions. When expanded, CO₂ immediately evaporates and the solute precipitates as a coprecipitate of the drug embedded in the polymer matrix. Various parameters that affect the resulting particle size and morphology are the pre- and postexpansion temperature and pressure, nozzle geometry, and solution concentration. The disadvantages of this method include the use of higher temperatures to form homogenous precipitates (thus degrading thermally labile drugs) and the limited solubility of the polymers and drugs that result in low drug loading[^35].

(5) **Phase Separation in Aqueous System**: This technique depends on the precipitation of the drug-entrapping polymer either by the addition of a third compound to the polymer solution or by some other physical means. The point has to be reached where two liquid phases are formed, the polymer-rich coacervate and the supernatant liquid phase, which is depleted in the polymer. Briefly, two steps are involved in the process: (i) the formation of liquid droplets of the polymer from the complete solution phase, which depends on the solubility parameters of the polymer, and (ii) subsequent hardening of the polymer droplets due to extraction or evaporation of the polymer solvent. A number of organic solvents, such as dichloromethane, isopropanol, and heptanes, have been used as solvent, coacervating agent, and hardening agent. If a drug is initially dispersed in the polymer solution, it can be coated by the coacervate. Phase separation could occur as a result of changes in pH or counterions or as a result of the aqueous phase acting as a nonsolvent for the polymer. Both hydrophilic and hydrophobic drugs can be entrapped. The main advantage of phase-separation method is that it protects active drugs from partitioning out into the dispersed phase[^36].

(5) **Spray Freeze Drying**: A typical Spray Freeze Drying (SFD) technique involves the atomization of an aqueous drug solution via a two-fluid or an ultrasonic nozzle into a spray chamber filled with a cryogenic liquid (liquid nitrogen) or halocarbon refrigerant such as chlorofluorocarbon or fluorocarbon. The spraying process can be performed beneath (spray-freezing into liquid) or above the surface of the cryogenic liquid, depending on the position of nozzle. It is also possible to use a nozzle arrangement for introducing liquid nitrogen directly into the spraying solution although the application of such a method for inhaled particles has
not been discussed. Since the level of the cryogenic liquid will inevitably drop due to evaporation, continuous addition of fresh cryogenic liquid is required, especially when a lengthy atomization process or a large spray volume is used. Upon contact with the cryogenic medium, the liquid droplets solidify rapidly (in milliseconds time scale) because of the high heat-transfer rate. Stirring of cryogenic liquid may be required to prevent the possible aggregation of newly formed frozen particles. Once the spraying process is completed, the whole content can be lyophilized, as with conventional freeze-drying\textsuperscript{[37]}.

**TOXICITY OF INHALED NANOPARTICLES**

With respect to the pulmonary route of drug delivery, toxicologists are primarily interested in the mechanisms by which smaller sized inhaled nanoparticles can cause lung injury and inflammation\textsuperscript{[38]}. When comparing the surface area of larger sized particles and very small sized nanoparticles the surface area of the smaller sized nanoparticles has been shown to promote interactions with biological systems leading to plausible negative effects\textsuperscript{[38]}. Specifically, it has been shown that nanoparticles have a larger inflammatory potential per unit mass compared to particles of larger sizes within rat lungs, however experiments pertinent to the toxicity of nanoparticles have mainly been investigated in vitro and only few in vivo studies are found in the literature\textsuperscript{[28]}. The primary means by which nanoparticles induce toxicological effects is through the production of reactive oxygen species thereby causing oxidative stress within the biological system. To fully explain the role of oxidative stress this process in pulmonary toxicity, the hierarchical oxidative stress model has been proposed\textsuperscript{[38]}. According to this model, reactive oxygen species (ROS) are produced as by-products during normal cellular respiration. Cells contain sufficient antioxidants such as glutathione and antioxidant enzymes and are thus able to neutralize these reactive species. In the case of severe lung injury (which might also be caused by inspiration of very small nanoparticles), the amount of ROS is greatly increased and consequently the cells do not contain enough antioxidants. This results in the accumulation of reactive molecules such as oxidized glutathione within the cells. Production of these ROS is also known to increase within the mitochondria in the presence of nanoparticles. An accumulation of oxide ions (O$_2^−$) is believed to be the consequence of particles becoming stalled within the mitochondria, hindering the electron transport chain and subsequent energy production. In such a situation, cells are able to detect the low ratio of glutathione to oxidized glutathione and subsequently induce inflammation\textsuperscript{[38]}. 
Experiments were conducted by Oberdörster et al. (Oberdörster, 1995) displaying the importance of the effect of surface chemistry on particle toxicity. An in vivo study was performed by exposing rats to polytetrafluoroethylene (PTFE) fumes which were heated to 480 °C. The application of heat resulted in the formation of PTFE nanoparticles with a median diameter of 18 nm. It was observed that inhalation of these fumes caused severe acute lung injury. Furthermore, high rates of mortality were found within four hours post inhalation. A subsequent study by Oberdörster explored the impact of inhaled ultrafine and fine titanium oxide (TiO2) particles in the lungs of rats and mice. Introduction of ultrafine (20 nm) TiO2 particles within the respiratory tract resulted in an exaggerated inflammatory response, determined by the increased number of neutrophils present within the lungs after 24 hours. Interestingly, exposure of the animals to an equivalent dose of fine sized TiO2 particles (250 nm) did not result in the exaggerated immune response that was observed with the ultrafine TiO2 particles thus emphasizing the importance of particle size for toxicity. Further data analysis revealed that the response elicited by both ultrafine and fine TiO2 particles was similar upon comparing their surface areas (rather than mass) to the percent of neutrophils found within the lungs. The results of the study served to indicate the notion that surface area is directly correlated with toxicity and may be employed to predict pulmonary toxicity.

APPLICATIONS OF PULMONARY NANOPARTICLES

Due to rapid advances in nanotechnology and biotechnology, nanoparticles have been considered as an effective form for delivery of the new generation of proteins and gene based macromolecular therapeutic agents into the body, since many of the components of living cells are constructed at the nano-level, such as ribosomes, membrane transporters, receptors and cell signaling systems. Nanoparticles fall in the same size range of the biological entities; therefore they can readily interact with molecules on both the cell surface and within the cell. Drugs that are deposited within the lungs in the nanoparticulate form have a greater chance to escape from the clearance mechanisms by the lung defense systems, compared to microparticulate form. Thus, drug bearing nanoparticles have the potential to deliver the drugs efficiently to the epithelium, while avoiding unwanted mucociliary clearance. Nanoparticles are useful to deliver water-insoluble drugs. Despite high potency, the effectiveness of water insoluble drugs can be severely limited because the solubility is too low to reach therapeutic systemic concentrations. However when their size is reduced to nano-level, the increased particle surface to volume ratio helps to enhance solubility and dissolution rate in an aqueous
environment. Nanoparticulate forms of drug could have an enormous benefit by significantly improving systemic bioavailability and allowing a more rapid onset of therapeutic action\cite{40}. The therapeutic applications of nanoparticles in respiratory and systemic diseases are numerous. Recent research has been focused on determining nanoparticles of different types to serve as vectors for the pulmonary drugs or genes delivery through inhalation or systemic administration, whereas various efforts have been undertaken for developing and delivering drug particles of nano-sized to the lung. Most of the studies reported have focus on the use of these strategies for the treatment of pulmonary infection. For example, transfer of gene using intranasal administration of chitosan-DNA nanospheres was shown to inhibit respiratory syncytial virus infection and to reduce allergic inflammation of airway in mice when given prophylactically or therapeutically. Moreover, nanoparticle-mediated intranasal delivery of short interfering RNA (siRNA) is required to target against a specific viral gene, NS1 which has also been shown to inhibit respiratory infection of syncytial virus in mice and rats). The significance of nano-sized drug particles for treatment of pulmonary infection has also been investigated. Aerosolized nano-sized itraconazole as inhalation resulted in significantly high lung concentrations, prophylactically inhibit invasive pulmonary aspergillosis and reduce infection-related deaths in mice, whereas oral drug administration did not. Pandey et al.\cite{41} demonstrated that a single inhalation of aerosolized poly (DL-lactide-co-glycolide) nanoparticles loaded with antitubercular drugs (isoniazid, rifampicin, or pyrazinamide) provided therapeutic plasma drug concentration for up to 6 days in guinea pigs and investigated that repeated inhalations were almost similar to oral administrations of free drug for treatment of experimental tuberculosis.

Anti-tubercular drugs have been successfully entrapped and delivered in biodegradable and biocompatible polymers. Zahoor et al.\cite{42} have developed inhalable alginate nano-particles as antitubercular drug carriers against experimental tuberculosis. The relative bioavailability of all drugs from the formulation have found significantly higher compared with oral free drugs when tested in guinea pigs. In another study, Justo et al.\cite{43} prepared the kanamycin-loaded lipid vesicles by ethanol injection method for administration by inhalation route. The selected drug was indicated for multiresistant tuberculosis, and administration through inhalation allows both local delivery of the drug to the lungs and systemic therapy. In a study by Garcia-Contreras et al.\cite{44} reported systemic delivery of insulin administered by the pulmonary route. The insulin formulations were administered by intratracheal instillation, spray instillation, and subcutaneous route. The plasma concentration of insulin and glucose were determined
and pharmacokinetic analysis suggested that the drug had longer mean residence time when administered to the lungs of Sprague-Dawley rats. Glucocorticoids such as budesonide, triamcinolone acetonide, and fluticasone, have a high degree of hepatic first-pass inactivation of the swallowed fraction of the inhaled dose, whereas there is no evidence of first-pass metabolism of these drugs in lung and when administered by inhalation are effective and widely used as anti-inflammatory agents of patient with asthma allergic rhinitis and advanced chronic obstructive pulmonary disease.

CONCLUSIONS
The pulmonary route of drug delivery has several advantages when compared to other routes for drug administration and benefits include a large surface area, extensive vascularization, avoiding first pass metabolism, and presence of a thin epithelial barrier. Research within the pharmaceutical industry towards the pulmonary route for drug delivery is gaining momentum and persistently being investigated for site-specific drug delivery of inhaled particles. Nanomedicine is gaining interest within the drug delivery field. Through the use of nanoparticles (particles with a mean diameter below 300 nm) it is expected that there will be improvements in site-specific drug delivery which will minimize side-effects resulting from the use of nonspecific drug carriers. Deposition of drugs to specific areas within the lungs may be enhanced and improved through the use of appropriate sized nanoparticles and may prove instrumental for treatment of pulmonary conditions such as asthma, chronic obstructive pulmonary diseases, lung cancer, tuberculosis and cystic fibrosis etc. With the perfection of pulmonary nanoparticle drug formulations, the lungs may become a preferred route of drug delivery for many local and systemic therapeutic agents.

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REFERENCES


