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Nanoparticles: A Novel Pulmonary Drug Delivery System for Tuberculosis

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Abstract
Tuberculosis or TB is a common and in many cases lethal infectious disease caused by various strains of mycobacteria. This article mainly focuses on the development of Nano particle systems and methods for delivering anti-tubercular drugs directly to the lungs via the respiratory route. The main advantages of inhaled drug delivery include direct drug delivery to the diseased organ, targeting to alveolar macrophages, reduced risk of systemic toxicity and improved patient compliance. Researchers have demonstrated the possibility of various drug delivery systems using lipids, polymers and proteins to serve as inhalable anti-tubercular drug carriers. In recent years, encapsulation of antimicrobial drugs in Nano particle systems has emerged as an innovative and promising alternative that enhances therapeutic effectiveness and minimizes undesirable side effects of the drugs. Beginning with the respiratory delivery of a single anti-tubercular drug, it is now possible to deliver multiple drugs simultaneously with a greater therapeutic efficacy. Several key issues such as patient education, cost of treatment, stability and large scale production of drug formulations, etc. need to be addressed before anti-tubercular inhaled therapy finds its way from theory to clinical reality.

INTRODUCTION TO TUBERCULOSIS:

Tuberculosis or TB (short for *tubercle bacillus*) is a common and in many cases lethal infectious disease caused by various strains of mycobacteria, usually *Mycobacterium tuberculosis*(MTB), is a small aerobic non-motile bacillus. Due to its High lipid content the pathogen accounts for many of its unique clinical characteristics. It divides for every 16 to 20 hours, an extremely slow rate compared with other bacteria, which usually divide in less than an hour. Since MTB has a cell wall that lacks a phospholipids in outer membrane, so it is classified as a Gram-positive bacterium. In nature, the bacterium can grow only within the cells of a host organism, but *M. tuberculosis* can be cultured *in vitro*. Tuberculosis usually attacks the lungs but can also affect other parts of the body.

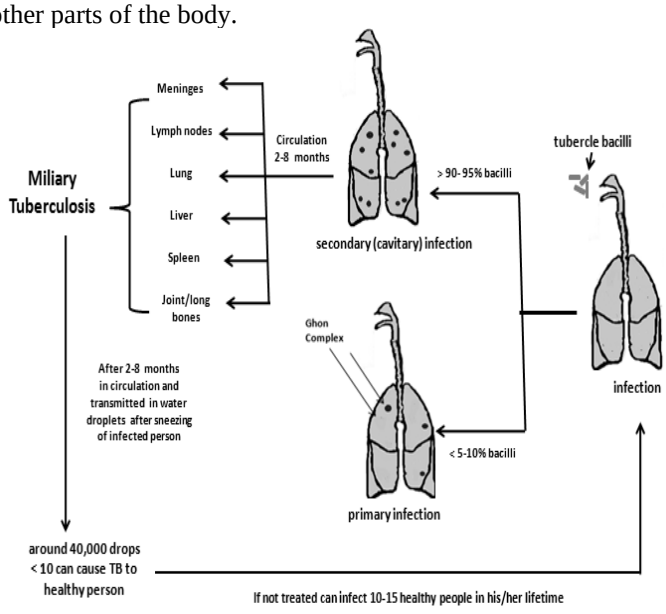


Figure-1 shows how the disease spreads through lungs

Treatment:

Treatment for TB uses antibiotics . Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacteria cell wall, which makes many antibiotics ineffective and hinders the entry of drugs. The two antibiotics most commonly used are isoniazid and rifampicin. However, instead of the short course of antibiotics typically used to cure other bacterial infections, TB requires much longer periods of treatment (around 6 to 24 months) to entirely eliminate mycobacteria from the body. Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance.

Pulmonary Drug Delivery:

Targeting drug delivery into the lungs has become one of the most important aspects of systemic or local drug delivery systems. Pulmonary drug delivery is a technology in which medicines are inhaled through the lungs and enters the bloodstream through the alveolar epithelium. It provides a noninvasive, alternative method to subcutaneous injection, and also intravenous injection. The delivery device plays a major role in the efficiency of pulmonary delivery, and great progresses have been made. The devices most commonly used for respiratory delivery, including nebulizers, metered-dose inhalers, and dry powder inhalers, can all is adapted for use with protein/peptide drugs. The choice of device will depend on the drug, the formulation, the site of action, and the pathophysiology of the lungs. Consequently, in the last few years, techniques and new drug delivery devices intended to deliver drugs into the lungs have been widely developed. The delivery by inhalation uses the extensive surface area of the alveoli, avoiding hepatic first-pass metabolism and enabling noninvasive administration of larger doses to the lungs, leading to greater therapeutic efficacy without

increasing toxicity. Pulmonary drug delivery is the preferred route of administration of aerosolized drugs in the treatment of respiratory diseases including asthma and cystic fibrosis, infectious diseases, in particular tuberculosis, and some nonrespiratory diseases such as type I diabetes.

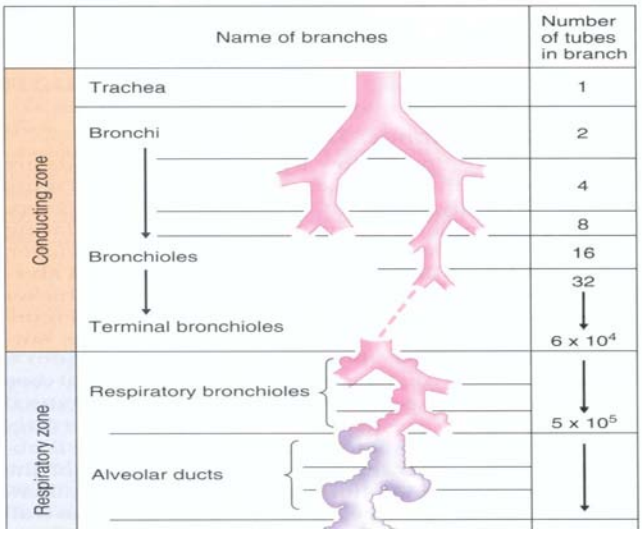


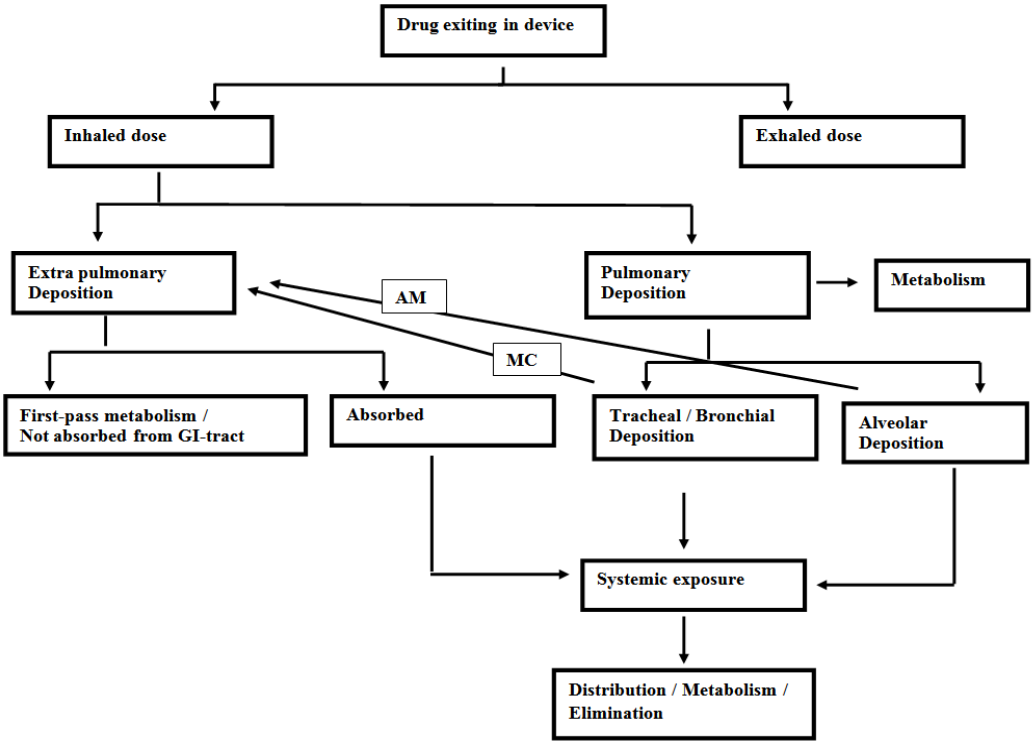
Figure-2 structure and functions lungs

Structure and function of the lung:

The human respiratory system can be divided in two functional regions 1) the conducting airways and 2) the respiratory region. The conducting airways, which are composed of the nasal cavity and associated sinuses, the pharynx, larynx, trachea, bronchi, and bronchioles, filter and condition the inspired air. From trachea to the periphery of the airway tree, the airways repeatedly branch dichotomously into two daughter branches with smaller diameters and shorter length than the parent branch. For each new generation of airways, the number of branches is doubled and the cross-sectional area is exponentially increased. The conducting region of the airways generally constitutes generation 0 (trachea) to 16 (terminal bronchioles). The respiratory region, where gas exchange takes place, generally constitutes generation 17-23 and is composed of respiratory bronchioles, the alveolar ducts, and the alveolar sacs. The number of times the bronchial tree branches before the gas exchange area is reached can vary from as few as 6 to as many as 28-30.

Absorption and bioavailability of inhaled drugs:

Pulmonary membrane is naturally permeable to small molecule drugs and many therapeutic peptides and proteins. Epithelium (barrier) is for absorption of drugs. The barrier thickness will decrease from trachea to alveoli. For large proteins, bioavailability and absorption mechanism are not completely elucidated. The change of cell type, morphology going from trachea, bronchi and bronchioles to alveoli is very dramatic. Natural mammalian peptides less than 30 amino acids are broken in lungs by ubiquitous peptidase and having poor bioavailability. Conversely proteins having molecular weights between 6000-50,000 Daltons are resistance to most peptidases and having good bioavailability. Greater fraction of drug deposition in alveoli or deep lungs shows higher bioavailability.



AM: phagocytosis by alveolar macrophages
MC: mucociliary clearance

Figure-3 Schematic illustration of the fate of an inhaled drug.

The more absorption of inhales drugs is due to drug efflux transporters and metabolizing enzymes are present in lungs in lower level than gastro intestine tract. Peptides are altered chemically to inhibit peptidase enzyme and exhibit higher bioavailability by this route (pulmonary). Small molecules can exhibit prolonged absorption if they are highly soluble or highly cationic. Very insoluble particles that slowly dissolve from inhaled particles may stick in lungs for many hours or even days. Fluticasone, propionate, amphothericinB and all Trans retinoic acids are absorbed from lungs over a period of hours due to the dissolution. Encapsulation in slow release particles such as Nano particles and liposome's used for controlling absorption. The bioavailability of proteins and peptides is 10-200 times more by pulmonary route when compared to other routes. Certain molecules that occurring on the lung lining fluid, they act as receptor mediated transport mechanism on alveolar epithelial cells having more absorption.

ADVANTAGES OF PULMONARY DRUG DELIVERY:

a. Inhaled drug delivery puts drug where it is needed.
b. It requires low and fraction of oral dose i.e. drug content of one 4 mg Tablet of salbutamol equals to 40 doses of meter doses.
c. Pulmonary drug delivery having very negligible side effects since. Rest of body is not exposed to drug.
d. Onset of action is very quick with pulmonary drug delivery.
e. Degradation of drug by liver is avoided in pulmonary drug delivery.
f. In asthma and diabetes requires long term treatment if it is given by pulmonary drug delivery safety is maximum because rest of body is not exposed to drug.

POLYMERIC NANO PARTICLES FOR ANTIMICROBIAL DRUG DELIVERY:

Polymeric Nano particles possess several unique characteristics for antimicrobial drug delivery. Firstly, they are structurally stable and can be synthesized with a sharper size distribution. Secondly, particle properties such as size, zeta potentials, and drug release profiles can be particularly tuned by selecting different polymer lengths, surfactants, and organic solvents during the synthesis. Thirdly, the surface of polymeric Nano particles typically contains functional groups that can be chemically modified with either drug moieties or targeting ligands. The initial polymeric Nano particles possessed poor therapeutic efficacy because of their rapid clearance by the recticuloendothelial system (RES) after intravenous administration. This limitation was overcome after the discovery of long-circulating polymeric Nano particles. For targeted antimicrobial delivery, polymeric Nano particles have been frequently coated with lectin, which is a protein that binds to simple or complex carbohydrates present on most bacterial cell walls. Due to their biocompatibility, surface modification capability, and sustained-release properties, polymeric Nano particles are intensively studied using various important pulmonary drugs. These pulmonary drugs include antiasthmatic drugs, antituberculosis drugs, pulmonary hypertension drugs and anticancer drugs. However, to avoid accumulation of polymer carriers following repeated dosing, the biodegradability and toxicity of polymers over the long term should be closely examined in the formulation of polymeric Nano particles for pulmonary delivery.

Protein Nano particles For Antimicrobial Drug Delivery:

Proteins are a class of natural molecules that have unique functionalities and potential applications in both biological as well as material fields. Among of colloidal systems those based on proteins may be very capable .Nano materials derived from proteins, especially protein Nano particles are biodegradable, non-antigenic, and metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. Because of the defined primary structure of proteins the protein-based Nano particles may suggest various possibilities for surface alteration and covalent drug attachment. Protein Nano particles can be utilized for the pulmonary delivery of protein therapeutics. The most important advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution as well as the improvement of the cellular uptake of a number of substances. As a result undesired toxic side effects of the free drug can be avoided.

Solid-Lipid Nano Particles for Antimicrobial Drug Delivery:

Solid-Lipid Nano particles are mainly comprised of lipids that are in solid phase at the room temperature and surfactants for emulsification. Solid lipid Nano particles (SLNs) are another antimicrobial drug delivery system. SLNs are typically particulate systems with mean diameters ranging from 50 nm up to 1000 nm for various drug delivery applications. Solid lipids utilized in SLN formulations include fatty acids (e.g. palmitic acid, decanoic acid, and behenic acid), triglycerides (e.g. trilaurin, trimyristin, and tripalmitin), steroids (e.g. cholesterol), partial glycerides (e.g. glyceryl monostearate and gylceryl behenate) and waxes (e.g. cetyl palmitate). Several types of surfactants are commonly used as emulsifiers to stabilize lipid dispersion, including soybean lecithin, phosphatidylcholine, poloxamer 188, sodium cholate, and sodium glycocholate. The typical methods of preparing SLNs include spray drying, high shear mixing, ultra-sonication, and high pressure homogenization (HPH). The advantages of drug release from SLNs in the lung are control of the release profile, achievement of a prolonged release and having a faster *in vivo* degradation compared to particles made from PLA or PLGA. In addition, SLNs proved to possess a higher tolerability in the lungs compared to particles made from some polymeric materials. Although SLNs for the pulmonary delivery is not fully appreciated, toxicological profile of SLNs, when using physiological lipids, is expected to be better than that of polymer-based systems, because physiological lipids have little or no cytotoxicicity. It is feasible that aqueous suspensions and perhaps dry powder formulations of SLN can be used for pulmonary inhalation.

Table-1 different types of polymers, proteins and lipids used for preparing Nano particles

Polymers	**Proteins**	**Lipids**
Polyesters	**Simple proteins**	**Non-digestible lipids**
Poly (Ethylene glycol) based Block Co Polymers	a) Albumins:- Blood(serum bumin); milk(lsctalbumin); egg white (ovolbumin) b) Globulins:- Blood (serum globulins); muscle (myosin); potato (tuberin); brazil nuts (excelsin); c) Glutens:- Wheat (glutenin); rice(oryzenin).	Mineral oils Sucrose polyesters
Polycaprolactones (PCL)	**conjugated proteins**	**Digestible lipids**
Poly (alkylcyanoacrylates)	a) Nucleoproteins:-cytoplasm of cell (ribonucleoprotein); nuclease of chromosomes(deoxyribonucleoprotein) b) Mucoproteins:- saliva (mucin); egg white (ovomucoid) c) Glycoprotein:- bone (ossemucoid); tendons (tendomucoid); d) Phosphoproteins:- milk (casein); egg yolk (ovovitellin)	**Triglycerides** Tricaprin Trilaurin Trimyristin Triopalmitin Tristearin
Poly (ortho-esters) (POE)	**Derived proteins**	**Diglycerides**
Polyanhydrides (PA)	a. Proteans:- edestan (elastin); mysoan (myosin) b. Proteases:- intermediate products of protein digestion c. Peptones:- same as proteases	Fatty acids
Polyamides		
poly (lactic acid)		
poly (glycolic acid)		
Poly (lactide-co-glycolide).(PLGA)		

PREPARATION OF NANO PARTICULATE INHALATION AEROSOLS:

In formulation preparation, several processing technologies have been used to obtain Nano particles for use as pulmonary inhalation aerosols with desirable attributes, such as narrow Particle size distribution, enhanced stability, controlled and targeted release, and improved bioavailability.

Spray-drying:

In this process, the feed solution is supplied at room temperature and pumped to the nozzle where it is atomized by the nozzle gas. The atomized solution is then dried by preheated drying gas in a special chamber to remove water moisture from the system, thus forming dry particles. These prepared particles are collected with a cyclone separation device. The spray-drying process is suitable for thermo labile materials such as proteins and peptides, because mechanical high energy input is avoided in this process. Colleagues replaced the bag-filter unit of a spray-drying system with a vacuum to reduce the drying airflow resistance. It allows the protein (recombinant humanized anti-lgE antibody) to be dried at a much lower temperature than usual and the production scale to be increased. In another study, poly(lactic-co-glycolic acid) particles containing proteins were successfully dried by ultrasonic atomization of feed solution into an atmosphere under reduced pressure. Solution spray-drying ensures compositional homogeneity of the drug powder, since the drug and the excipients are dissolved prior to the process.

Spray-freeze-drying (SFD):

Spray-freeze-drying (SFD) is an advanced particle engineering method which combines spray-drying and freeze-drying processing steps. This technique involves the atomization of an aqueous drug solution into a spray chamber filled with a cryogenic liquid (liquid nitrogen)or halocarbon refrigerant such as chlorofluorocarbon or fluorocarbon. The water is removed by sublimation after the liquid droplets solidify. SFD is capable of producing porous particles with high fine particle fraction (FPF) at sub ambient temperatures. They are usually low-density composite amorphous particles with high specific surface area. Thermo labile protein and peptide substances, such as insulin and plasmid DNA, can also be formulated into dry powder inhalation products by SFD

Supercritical fluid technology (SCF):

This method has demonstrated a wide range of application in producing pulmonary inhalable formulations. The basic feature of the supercritical fluid process is the controlled crystallization of drugs from dispersion in supercritical fluids such as carbon dioxide. Two most important technologies are supercritical antisolvent precipitation (SAS) and supercritical fluid extraction of emulsions (SFEE). The fundamental mechanism of SAS is based on rapid precipitation when a drug solution is brought into contact with a supercritical CO_2. SFEE is based on extraction of the organic phase in oil-in-water or multiple emulsions using supercritical CO_2. Because most of the drugs (eg, asthma drugs) are not soluble in CO_2, SAS processes provide an easy and excellent way to produce dry powder inhalation formulations. SFEE can provide uniform crystalline drug nano particles, composite nano particles containing polymeric materials and the drugs, and nano suspensions. For instance, Chattopadhyay and colleagues used a continuous SFEE method to produce nanoparticle suspensions containing one of three lipids (tripalmitin, tristearin, or gelucire 50/13), and one of two model drugs (indomethacin or ketoprofen). The first step of this process was to produce nanoemulsions by mixing organic

phase containing lipids, a selected drug and chloroform with aqueous phase containing sodium glycocholate, under high pressure homogenization. Then the nanoemulsions were introduced to an extraction chamber countercurrently to a stream of supercritical CO_2. The CO_2 extracted the organic solvent from the dispersed droplets, forming nanoparticles with a volume mean diameter between 10–30 nm with a high drug loading efficiency for the gelucire particles (80%–90%).

Double emulsion/solvent evaporation technique:

Respiratory nanoparticles formation from double emulsion/ solvent evaporation system involves preparation of oil/water (o/w) emulsions with subsequent removal of the oil phase (ie, typically a volatile organic solvent) through evaporation. The emulsions are usually prepared by emulsifying the organic phase containing the drug, polymer and organic solvent in an aqueous solution containing emulsifier. The organic solvent diffuses out of the polymer phase and into the aqueous phase, and is then evaporated, forming drug-loaded polymeric nanoparticles. By this method, biodegradable polymers, including poly (l-lactic acid) (PLA), poly (glycolic) acid (PGA), and poly (lactide-co-glycolide) acid (PLGA), have been intensively investigated as carriers for solid drug nanoparticles.

Antisolvent precipitation:

Crystalline drug particles with narrow size distribution could be prepared by direct controlled crystallization. This process involves antisolvent precipitation of drug solution in a water-miscible organic solvent, followed by addition of a bridging solvent, which is immiscible or partially miscible with water. Growth-retarding stabilizing additives, such as hydroxylpropylmethylcellulose (HPMC), are usually added in the medium to yield particles with small size. The precipitated drug crystals exhibit a high FPF and low amorphous content.

CONCLUSION

As more efficient pulmonary delivery devices and sophisticated formulations become available, physicians and health professions will have a choice of a wide variety of device and formulation combinations that will target specific cells or regions of the lung, avoid the lung's clearance mechanisms and be retained within the lung for longer periods. In conculation,Nanoparticles are the more efficient, user-friendly delivery devices may allow for smaller total deliverable doses, decrease unwanted side-effects and increase clinical effectiveness and also improve patient compliance, which may satisfy the above expected need in pulmonary delivery especially Tuberculosis or TB.

Table-2 different devices used for pulmonary drug delivery

Device	Advantage	Disadvantages
Nebulizers	No specific inhalation technique is required Delivers large doses Suitable for infants	Time consuming Poor delivery efficiency Drug lost Relatively expensive
Pressurized metered dose inhalers (pMDI)	Compact Multi-dose Portable inexpensive	High oral deposition Maximum dose of 5mg
Dry powder inhalers(DPI)	Compact Portable Easy to use	Respirable dose dependent on inspiratory flow rate Dose lost if patient inadvertently exhales into the DPI

Table-3 Characterization of Nano particles

Type	Method	Measurement
In-vitro	Dissolution test Inertial impaction Delivered dose assay Laser diffraction Laser droplet velocimetery	Dissolution rate Aero dynamic particle size distribution (APSD) Total delivered dose and dose uniformity Particle size and distribution Aerosol velocity
In-vivo	Scintigraphy Pharmacokinetic (PK) —animal model/clinical trial Phamacodynamics (PD)	Visualization and quantification of aerosol deposition in respiratory tract PK parameters Biochemical and physiological effects of a drug
Ex-vivo	Isolated perfused lung	Mechanisms of drug transport and disposition

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