Recent Advancment in Nanoparticles for Pulmonary Drug Delivery System

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Abstract

Recent advancements in nanoparticle-based pulmonary drug delivery systems have garnered significant attention due to their capacity to enhance therapeutic efficacy and safety. The distinctive properties of nanoparticles enable precise targeting, regulated drug release, and enhanced bioavailability within the lungs. This review provides a comprehensive overview of recent breakthroughs in nanoparticle-based pulmonary drug delivery, encompassing various nanoparticle types, fabrication methods, and physicochemical characteristics that influence drug delivery outcomes. Additionally, we examine the challenges and opportunities involved in translating these systems from laboratory to clinical settings. This review underscores the vast potential of nanoparticles to revolutionize pulmonary drug delivery and emphasizes the need for continued research and development in this field."

***Keywords*:** nanoparticles, SLN (solid lipid nanoparticle), lung cell models, aerosol, isolated perfused lung (IPL), precision cut lung slices (PCLS)

I. Introduction

The lungs are an ideal target for the administration of active pharmaceutical ingredients (APIs) via pulmonary drug delivery systems [1-3]. Furthermore, pulmonary administration offers several advantages over traditional oral delivery, including rapid absorption due to high vascularization, a large surface area, and avoidance of the first-pass effect [2]. "This targeted approach allows for selective drug delivery, reducing side effects. Colloidal drug delivery systems have extensively been investigated as drug carrier systems for the application of different drugs via different routes of administration. Solid lipid nanoparticles (SLN) are one of the most interesting colloidal systems that has studied for more than a decade[4-7]. Solid lipid nanoparticles (SLN) are aqueous nanoscale suspensions composed of phospholipids and triglycerides that are well-tolerated by the body [8-10]. In addition to SLN, biodegradable polymeric nanoparticles are gaining recognition for their ability to release APIs in a sustained manner. These systems are particularly well-suited for lipophilic drugs, which have limited solubility in water. By increasing solubility in a lipophilic environment, these systems enhance pharmacokinetics and therapeutic efficacy. Moreover, nanoparticle-mediated drug delivery systems offer innovative solutions by tailoring particle properties, such as drug solubility, encapsulation efficiency, and surface characteristics, to optimize drug release profiles and achieve optimal therapeutic outcomes [11] .While nano systems offer therapeutic benefits, their potential toxicological effects must also be considered. To ensure safety, thorough toxicological testing is necessary across various cell culture models, including in vitro, ex vivo, and in vivo studies, to determine a safe dosage. Although results from cell culture models may not directly translate to individual patient outcomes, such testing is crucial to minimize the risk of adverse reactions or toxic effects. Furthermore, the selection of an appropriate inhalation device for a specific patient population is critical in nanoparticle-mediated drug delivery systems for pulmonary applications [12,13]. The various nanoparticle systems and various parameters to be considered during formulations developed is illustrated in **figure 1**
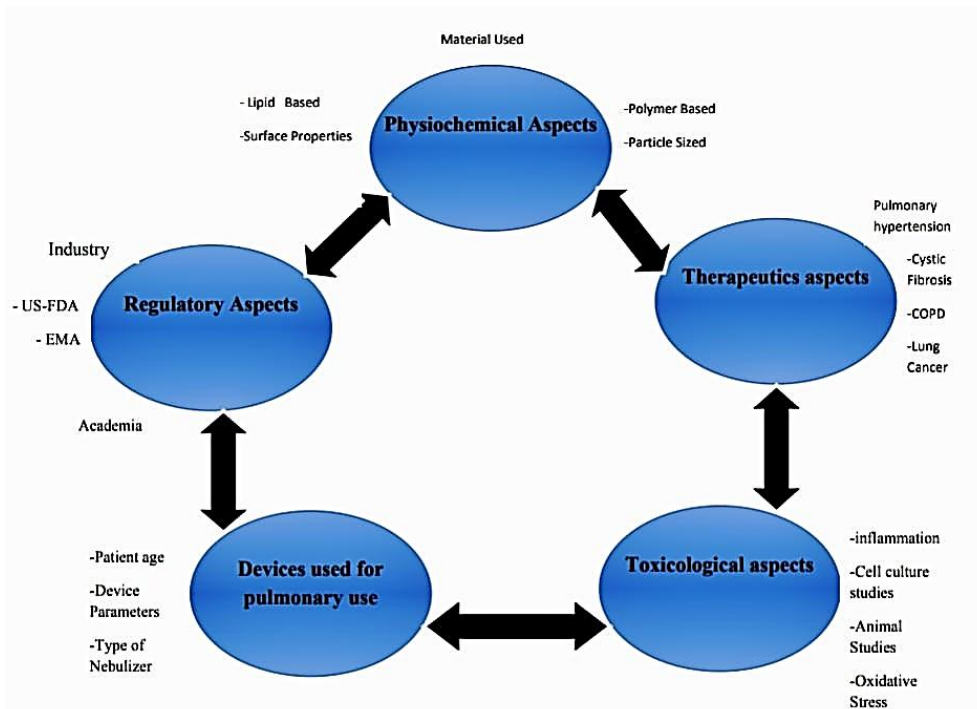
Figure 1. complex interplay of parameters in the research and development of pulmonary drug delivery systems.

1.1 Anatomy And Physiology Of The Lung
1.1.1 Anatomy of lungs

The lungs, comprising five lobes (three in the right lung and two in the left), are responsible for exchanging gases and supplying oxygen to the body's cells. The interior features of lungs is a complex network of bronchi, smaller air passages, alveoli, blood vessels, and lymph tissue. The bronchi are further divided into primary and secondary bronchi and bronchioles and, finally, the alveoli. Lungs have over 300 million alveoli. Moreover, each alveolus is lined with pulmonary capillaries, thus forming a vast network comprising over 280 billion capillaries. the lungs boast an expansive surface area of approximately 70 square meters for gas exchange. This process primarily occurs at the interface of alveolar epithelium, endothelium, and interstitial cell layers. The alveolar wall consists of two types of alveolar epithelial cells (Type I and II pneumonocytes). Between the capillary and alveolar epithelium there exist a single endothelial layer, facilitating diffusion due to the extremely thin (0.5 µm) blood-gas interface. A layer of alveolar fluid and mucus, rich in phospholipids and surface proteins, coats the alveoli, reducing surface tension and enabling efficient gas exchange. Supported by a thin layer of connective tissue, these distal respiratory passages are surrounded by various cells, including macrophages, fibroblasts, nerves, and lymph vessels, making them an ideal location for drug administration targeting both the pulmonary and lymphatic systems.[14,15]
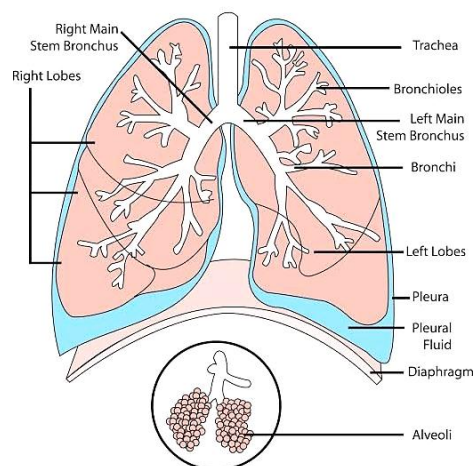

Figure 2 ; Anatomy of human lung [67]

1.1.2 Deposition of the particle

The formulation's particle size determines how much material is deposited in each area of the lungs. Three distinct drug deposition mechanisms impaction, sedimentation, and diffusion are identified based on particle size [16]. The aerosol particles move quickly through the oropharynx and upper respiratory passage during impaction with high velocity. The particles are deposited in the oropharynx areas after colliding with the respiratory

wall as a result of centrifugal force. With particles larger than 5 m, this process is typically seen in dry powder inhalation (DPI) and metered dosage inhalators (MDI). The patient's breathing is crucial to the deposition in the case of DPI. Due to the mass of the particles and the inertial forces, dry powder will deposit in the upper airways if the force of inhalation is insufficient. fast particle sizes also likely to cause the deposition of the particles, especially in the upper respiratory tract region, for the MDI, despite the fast speed of the created aerosol. The majority of the time, gravitational forces causes particles to settle. When given enough time and adequate mass, particles with sizes between one and five micrometers are deposited in the bronchioles and smaller airways, where they are slowly deposited. As a result, breathing style also affects sedimentation. A sufficient period of slow breathing allows for sedimentation. In the deeper alveolar regions of the lungs, Brownian motion is important in addition to impaction and sedimentation. The particles travel at random due to the Brownian motion of the molecules surrounding the aqueous lung surfactant. The dissolution of API in alveolar fluid upon contact with the lung surfactant is crucial for diffusion. Additionally, the diffusion process is impacted by the concentration gradient. Most of the particles, because of their lower sizes, are exhaled, with the exception of those smaller than one to 0.5 m, which are deposited in the alveolar region [17]. Sedimentation is the most desirable mode of particle deposition for nanoparticulate systems. When released from an aerosol, nanoparticulate systems form micrometer-sized aggregates. It is believed that these aggregates have sufficient mass to sediment and remain longer in the bronchiolar region, achieving the desired effect. Aside from the instruments, boundaries, for example, the molecule size of the spray, molecule morphology and math, alongside surface properties, assume a significant part in statement peculiarities. The deposition is also affected by humidity, air velocity, and tidal volume, as well as breathing frequency and holding breaths [17].

Location	Size	mechanism
Primary Bronchi	5-10 μm	Impaction
Secondary Bronchi	1-5 μm	Sedimentation
Bronchioles	1-3 μm	Sedimentation
Alveoli	0.5-1 μm	Brownian motion

Table-1 shows the relationship between drug deposition area and particle size [68]

1.1.3 Clearance of the particle

A protective mucus barrier in the upper airways, extending from the trachea to the tertiary bronchi, traps particles and prevents their entry. Mucociliary movements swiftly eliminate these particles, preventing their advancement to the lower lungs through coughing or swallowing [18,19]. The clearance process in this region is influenced by mucus characteristics, cilia number, and ciliary beat rate [16]. However, the transport mechanism in the alveolar region, located deeper in the lungs, is thought to be more intricate. The alveolar lining, composed of various proteins and lipids, acts as a barrier that restricts molecular movement. The tight junctions between epithelial cells, along with the alveolar lining, form the primary obstacle to transport. The role of transporter proteins is critical, depending on the API's properties and chemical structure, in facilitating either active absorption or passive diffusion. Another crucial factor in this region is the removal of molecules by alveolar macrophages, which must be considered in drug transport mechanisms [16,19]. Molecules that successfully cross the barrier are either absorbed into the systemic circulation through cellular uptake or engulfed and eliminated by alveolar macrophages through phagocytosis.

Therefore, understanding the physiology of the lungs is essential for comprehending the drug formulation's uptake and clearance mechanisms. There is still a lack of information regarding the precise uptake, transport, and clearance of particles in the alveolar epithelium and the method by which API molecules reach the systemic circulation despite advancements in formulation development. Albeit a few in vitro models have been laid out for concentrating on the take-up and penetration of the APIs in the pneumonic epithelium (air-fluid connection point models), there are as yet open inquiries concerning the way of behaving of the cells in a sick condition. The exchange of various cell types required alongside the affidavit systems is represented in **Figure 3**
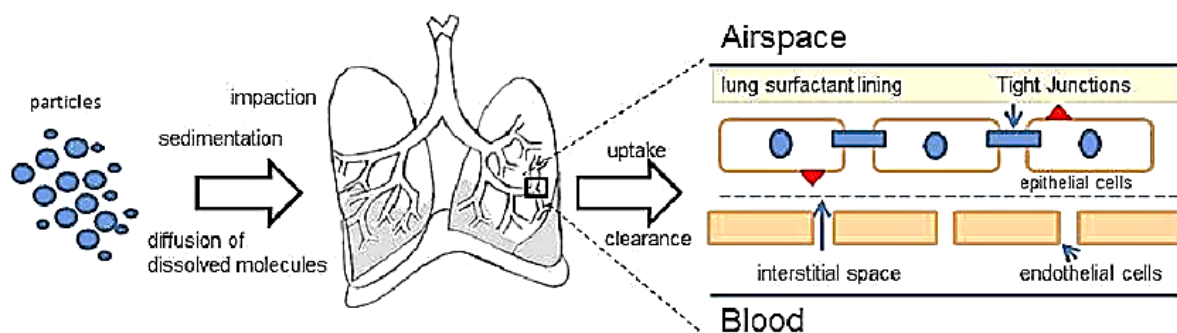
Figure 3- The deposition mechanism and uptake of particles in the lung along with different cell types [68]

Being in contact with the air, the lungs are vulnerable to a wide range of illnesses and conditions, from respiratory infections to genetic and lifestyle diseases. The most prevalent conditions include chronic lung malignancies, pulmonary hypertension, acute respiratory distress syndrome (ARDS) in babies, cystic fibrosis, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) in adults, and asthma.

II. Nanoparticle -Based System For Pulmonary Applications

2.1.1 Lipid Based Nanoparticles (Liposome, Solid Lipid Nanoparticles, Nano-Emulsions)-

Lipid-based nanoparticles, specifically solid lipid nanoparticles (SLN), have been extensively researched for their potential in delivering pulmonary medications over a prolonged period. SLN (solid lipid nanoparticles) are composed of physiological lipids, including triglycerides and phospholipids, which are formulated into nanoscale aqueous suspensions. The use of physiological components in these formulations reduces toxicity, making them more suitable for pulmonary medication administration. The lungs' deep regions are rich in phospholipids, which play a vital role in maintaining the respiratory system's proper function. Phospholipid-based surfactant proteins are essential at the alveolar surface to maintain optimal surface tension and minimize lung tissue friction [20] . Various medications have been investigated for treating lung infections, including the development of amikacin-loaded SLN using cholesterol and high-pressure homogenization, which is an amino glycoside antibiotic [21]. The investigation has been done for the biodistribution of amikacin solid lipid nanoparticles (SLN) after pulmonary administration. To track the drug's tissue deposition, they used radioisotope technetium (99mTc) labeled amikacin. In vivo experiments involved administering radio-labeled amikacin or placebo to rats via pulmonary and intravenous routes. Gamma scintigraphy studies revealed that pulmonary administration of 99mTc-amikacin SLN resulted in a longer lung retention time compared to intravenous administration. Moreover, the lung deposition of 99mTc-amikacin SLN was significantly higher than in the kidneys. These findings highlight the value of radio labeling assessments using gamma scintigraphy in obtaining detailed information on API tissue deposition. Conducting such studies in animal models with diseased conditions would provide more accurate data. Additionally, certain fatty acids can enhance drug solubility and biodistribution, further improving the efficacy of SLN [22].

2.1.2 Polymeric nanoparticles (poly (lactic-co-glycolic acid), chitosan, gelatin)

Polymers are gaining rapid importance for pulmonary drug delivery. Several polymers have been investigated for pulmonary application. Polymers have numerous advantages, like modified surface properties, high encapsulation of the drug and protection of the drug from degradation, prolonged drug delivery and a long shelf life. For therapeutic purposes, the most commonly used polymers include poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), alginate, chitosan and gelatin base [11]. These are modified in their chemical and surface properties in order to make them biodegradable [23]. A recent study assessed the impact of polymeric nanoparticles on pulmonary surfactant and its surface properties. The research compared the effects of synthetic and biodegradable polymeric nanoparticles on the pulmonary surfactant, revealing dose-dependent changes in surface tension . They found dose-dependent changes in the surface tension of the pulmonary surfactant [23,24]. Several studies have been performed using chitosan-based nanoparticles for protein and gene delivery. Experiments were conducted to manufacture nanoparticles containing phospholipid and chitosan using the ionic gelation method at varying pH conditions for the pulmonary delivery of low molecular weight heparin. In vivo studies in mice revealed that nanoparticles prepared

in acidic conditions did not affect coagulation time, whereas those prepared in neutral conditions significantly increased coagulation time [25]. Recent studies have investigated the use of polymeric micelles and nanoparticles for pulmonary drug delivery. One study combined polyethylene glycol (PEG5000) and poly(ethylene oxide)-block-distearoyl phosphatidyl ethanolamine (DSPE) to create paclitaxel-loaded polymeric micelles, which were administered through intratracheal instillation and intravenous injection in in vivo models. The results showed improved drug absorption and targeted drug delivery to the lung tissue compared to intravenous administration [26]. Another study used an amphiphilic block copolymer composed of poly(glycolide-caprolactone) with PEG and tocopheryl succinate to encapsulate paclitaxel, enhancing encapsulation efficiency and cellular uptake. The copolymer-paclitaxel was compared to a commercial taxol product and showed improved drug release patterns and reduced cytotoxicity[27,28].

2.1.3 liposomes

Liposomes offer a promising drug delivery system for pulmonary applications, leveraging their natural compatibility with lung tissue due to their phospholipid composition. Formulated with lung surfactants, phospholipids, cholesterol, and other components, liposomes exhibit sustained release properties, ensuring an extended and optimal drug effect. The first liposomal product, Alveofact®, was launched in the 1990s for the treatment of acute respiratory distress syndrome (ARDS) in infants, administered through pulmonary instillation. This was followed by the introduction of Ambisome®, a liposomal formulation of amphotericin B, which was approved for parenteral use, marking a shift away from pulmonary applications [29]. Despite the challenges, inhaled liposomes hold promise for treating lung infections. To achieve a successful product, it's crucial to preserve the liposomes' physical properties during nebulization. Currently, two dry powder liposomal products, Arikace® (amikacin) and Pulmaquin™ (ciprofloxacin), are in advanced clinical development stages, offering potential treatments for lung infections [30-33]. Arikace®, a liposomal amikacin formulation, is currently being evaluated in a Phase 2 trial for the treatment of Pseudomonas aeruginosa lung infections in cystic fibrosis patients. A recent double-blind, randomized study enrolled 105 patients with Pseudomonas. aeruginosa infections, who were randomly assigned to receive either once-daily amikacin aerosol or a placebo for 28 days [34]. The study results showed that amikacin treatment significantly reduced the density of Pseudomonas. aeruginosa in patient sputum and improved lung function compared to the placebo group. The findings demonstrated that amikacin was safe and well-tolerated, with improved lung function and reduced bacterial density in cystic fibrosis patients. Additionally, liposomal ciprofloxacin is being investigated for its potential in treating lung infections [34]. Liposomes loaded with ciprofloxacin were successfully prepared using phospholipids and cholesterol via the film method, exhibiting an average particle size of 350 nm and a high encapsulation efficiency of up to 93%. In vitro drug release studies revealed a significantly higher cumulative release of ciprofloxacin from liposomes in simulated lung fluid (SLF) compared to saline solution. Furthermore, in vivo experiments conducted in rats demonstrated enhanced drug targeting efficiency of ciprofloxacin-loaded liposomes compared to ciprofloxacin solution, highlighting their potential for targeted lung delivery [35]. Phospholipid-based liposomes containing antioxidants like n-acetylcysteine, vitamin E, and glutathione were produced using the film method. An in vivo rat study showed reduced pro-inflammatory cytokine levels in the bronchoalveolar lavage fluid (BAL) of CEES-injured rats. These antioxidant-containing nanoparticles can be delivered to the lungs via nebulization or instillation, offering potential therapeutic benefits for hypoxia and oxidative stress-related lung injuries. Liposomes, whether based on physiological lipids or polymers, in liquid or dry powder form, have long been considered promising candidates for pulmonary delivery of various active pharmaceutical ingredients (APIs) [36].

III. Pulmonary Targeting: Strategies for Nanoparticle Delivery

Nanoparticles alone can deposit in the lungs, but their delivery to the pulmonary mucosa is inefficient due to reliance on Brownian diffusion. Effective pulmonary drug delivery requires reliable and repeatable particle deposition, necessitating aggregation or incorporation of nanoparticles into a carrier to achieve an aerodynamic diameter of 1-5 mm for deep lung deposition. Various methods, such as particle aggregates, solid dispersions, or liquid dispersions, can accomplish this, each with advantages and disadvantages depending on the active ingredient, aerosolization mechanism, and therapeutic needs.

3.1.1 Nebulized dispersions : nanoparticles offer a promising method for pulmonary drug delivery, enabling uniform dosage delivery and efficient deep lung deposition. Compared to micronized drug dispersions, nanoparticles provide more even distribution and fewer detrimental effects on nebulizer function. However, the choice of stabilizing polymer or surfactant is crucial, as many synthetic options are toxic or immunogenic in the

lungs. Natural surface active agents or redispersion designs are often used to stabilize nano dispersions, and dry formulations may offer an advantage by requiring minimal surfactant [59]

a) Stabilized Nanodispersions- For the purposes of nebulization, surfactant stabilized colloidal dispersions have been created to aid in the administration of nanoparticulate drugs to the lungs. Dipalmitoyl phosphatidylcholine (DPPC), lecithins, and leucines are examples of natural surfactants that can be included in these formulations to ensure colloidal stability and avoid the agglomeration of dispersed particles. This formulation strategy has been used by inhalation researchers, who have discovered several aerosolization and physiological benefits [60]

b) Nano particle redispersion: To overcome instability issues, researchers developed nanoscale powders for redispersion, which can be dispersed before delivery, eliminating the need for stabilizing surfactants. This approach is particularly useful for therapeutics and polymeric carriers prone to hydrolytic degradation. By using lyoprotective substances like lactose, mannitol, glucose, and sucrose, powders can be reconstituted and nebulized while maintaining stability and particle size, making it a promising method for targeted and biopharmaceutical therapy.

c) Nanoscale Powders for Redispersion: To address potential instability, researchers developed a formulation for dispersion before delivery, avoiding the need for stabilized colloidal dispersions and surfactants. This approach is particularly useful for therapeutics and polymeric carriers prone to hydrolytic degradation. Formulations for redispersion have gained interest for targeted and biopharmaceutical therapy using nanoparticles as carriers [61]

d) Drug Encapsulated Nano dispersions : involve enclosing drugs in a carrier material to improve solubility, stability, and controlled release. Surface modification enables targeting and stealth features. Researchers created tumor-targeting nanoparticles for nebulization to achieve high lung concentrations of chemotherapeutic drugs while minimizing systemic exposure. In a study, surface-modified gelatin nanoparticles with biotinylated EGF showed preferential deposition in lung tissue of tumor-induced mice, accumulating in lung tissue without adverse effects, demonstrating potential for targeted lung cancer therapy [62]

e) Lipid nanoparticles are a promising formulation for inhalation medications due to their natural composition, which reduces pulmonary and systemic toxicity concerns. Liposomes, composed of saturated and unsaturated hydrocarbons similar to those found in human tissues and fluids, have gained attention for lung delivery. Many large-scale clinical trials have been conducted to explore liposomal delivery to the lungs, offering a technically nanoparticulate (200 nm) approach with potential benefits for lung medication administration [63]

3.1.2 Dry powders for inhalation offer a promising alternative to liquid nebulization for delivering nanoparticles to the lungs. Unlike liquid nanodispersions, which can be unstable due to hydrolysis, settling, or aggregation, dry powders provide superior chemical and physical stability. This is because the dry state reduces molecular mobility, minimizing degradation and agglomeration. Additionally, dry powder inhalers can deliver a single dose in a single breath, shortening treatment times and improving patient compliance. This makes dry powders a practical and convenient option for routine medical care.

a) Nanodisperse Microspheres: Microencapsulation is a formulation strategy used to control drug release, prevent degradation, and enhance bioavailability. For pulmonary administration, microencapsulation is used to create particles with the right aerodynamic diameter for deep lung deposition. Spray drying is a common method used to produce powders for inhalation, and researchers have explored this technique to incorporate nanoparticles into carrier micro particles [64]

b) Aggregated Nanoparticles: Depositing discrete aerosolized nanoparticles in the lower airways can be challenging due to the minor impact of inertial and sedimentation forces. Forming low-density aggregates from various nanoparticles is a successful method to enhance these forces on dry nanoparticulate aerosols. These nanoaggregates can be produced using methods like spray drying, salt flocculation, and quick freezing [65]

c) Nanoparticle Toxicology: Regulatory bodies and research organizations are concerned about the impact of insoluble nanoparticles on biological systems. The high surface area and bioavailability of nano-sized medicines can translate to toxicity issues, particularly when inhaled. Nanomaterials can cause immunobiological reactivity, prolonged tissue retention, and easily enter the bloodstream due to their small size, leading to toxicity concerns [66]

IV. Physiochemical Properties Of Nanoparticles;-

4.1.1 Particle Size and Zeta Potential Measurements:

Accurate characterization of nanoparticles requires measuring particle size and zeta potential to ensure optimal particle size distribution and polydispersity index (PDI). Common methods for particle size measurement include Photon Correlation Spectroscopy (PCS) and Laser Diffraction (LD).

PCS measures particle sizes ranging from a few nanometers to three millimeters by detecting light scattering caused by Brownian motion. It also measures PDI, which indicates particle uniformity. A lower PDI value (<0.2) indicates a more uniform particle distribution. LD measures larger particles by detecting diffraction angles based on particle radius, covering a size range from nanometers to a few millimeters. Using both methods simultaneously provides more accurate results. Zeta potential measurements predict particle stability. Higher zeta potential values indicate greater repulsion between particles, reducing aggregation and increasing shelf life. A more even particle distribution ensures better stability[,42,7].

4.1.2 Differential Scanning Calorimetry (DSC) :

Differential Scanning Calorimetry (DSC) is a crucial analytical technique for detecting polymorphic changes in lipid matrices. By examining structural changes in the lipid matrix, DSC provides valuable insights into the formulation's stability over time. Specifically, the melting and recrystallization curves obtained from DSC analysis serve as key indicators of polymorphic changes in lipid matrices, allowing researchers to assess the formulation's stability and potential for transformation into different polymorphic forms[37-39].

4.1.3 Microscopical Techniques/Particle Morphology ;-

The morphology of the nanoparticles can be examined by using transmission electron microscopy utilizing different techniques suitable for specific particles. Freeze-fracture, negative staining and cryogenic-transmission methods can be adapted according to the type of particles. The interpretation of the morphology of the particle gives an idea about the structure, shape and alignment of the particles in the formulation. The interpretation of the size can also be confirmed using this method [40].

4.1.4 X-ray Diffraction: Unveiling Crystal Structure and Lattice Spacing

X-ray diffraction, in conjunction with Differential Scanning Calorimetry (DSC), is crucial for determining the crystal structure and lattice spacing of lipid matrices. The addition of an Active Pharmaceutical Ingredient (API) alters the lattice spacing and lipid/polymer structure, making it essential to use both techniques simultaneously. This combined approach enables the mapping of crystallinity, revealing patterns in lattice spacing and changes in lipid and polymer structures. Advanced techniques for physicochemical characterization include, Nuclear Magnetic Resonance (NMR) spectroscopy, Raman spectroscopy, Infrared spectroscopy. These methods are valuable tools for analyzing complex systems, where multiple particle types coexist (e.g., Solid Lipid Nanoparticles (SLN), micelles, liposomes, and liquid crystals). They provide detailed information on the physical and chemical properties of these systems, enabling a deeper understanding of their behavior and interactions [41].

V. Cell- and Animal-Based Studies

Toxicity analysis of nanoparticle systems has been conducted across various cell lines, tissue models, and animal models to determine the lethal dose and therapeutic window of drug-loaded nanoparticles. In addition to toxicity testing, assessing therapeutic efficacy is crucial, and numerous models have been developed to mimic diverse disease conditions. In vitro models utilizing respiratory tract epithelial cells have been established to evaluate nanoparticles following inhalation, while air-liquid interface (ALI) models have been widely employed to investigate the effects of pulmonary formulations [43-47].

5.1.1. In Vitro Lung Epithelial Cell Culture Models

In vitro cell culture models are crucial for initial formulation testing, offering advantages like continuous cell lines, ease of handling, and availability in large numbers, which offers the user many possibilities for designing multiple experiments simultaneously and, hence, limits the use of live animals.. Pulmonary epithelium-derived cell models from human and murine tissues have been developed over the past two decades. The goal is to establish a standardized cell line that accurately predicts transporter mechanisms across the pulmonary epithelium, similar to the Caco-2 cell line used for gastrointestinal tract studies. Various studies have utilized pulmonary epithelial cell lines from human and murine sources as a lung equivalent to Caco-2. A human bronchial epithelium cell line (16HBE14o) has been used for a long time for studying the drug transport mechanism in airways. This cell line has been established as a model for studying airway transport and drug permeation mechanisms. Different experimental parameters, such as seeding density, transepithelial electric resistance (TEER), and culture conditions necessary for optimal drug transport, have been studied [48,49]. The barrier properties of the 16HBE14o cell model were further characterized by assessing the transport of mannitol and analyzing the transepithelial electrical resistance (TEER) values [50]. This analysis revealed that the 16HBE14o cell line exhibited increased permeability to hydrophilic molecules compared to traditional alveolar epithelial cell models. Moreover, a sigmoidal relationship was observed between the permeability and lipophilicity

of lipophilic molecules, indicating a non-linear correlation between these parameters.

5.1.2. Ex Vivo Lung Tissue Models

Ex vivo lung tissue models have been used extensively along with the in vitro and in vivo models. Ex vivo models can be advantageous for studying drug transport mechanisms across lung tissue and can provide information with respect to in vitro-in vivo correlation. Different ex vivo models were established, such as isolated perfused lung (IPL) and precision cut lung slices (PCLS).

IPL are prepared from mostly murine models, i.e., rats. Mice are seldom used, owing to their small size and the difficulty in the isolation procedure. Other animals commonly used include rabbits and guinea pigs. The IPL model includes a complete lung that has been isolated from the body and immersed in an artificial system that resembles physiological conditions. IPL is encased in a system containing physiological buffer solutions (Krebs–Ringer or Krebs–Henseleit), where a 37 °C temperature condition is maintained. The perfusate flow is maintained between 12–15 mL/min. The perfused solution is also equilibrated with a mixture of oxygen and carbon dioxide to ensure the proper functioning of the lung tissue. The advantage of the IPL model is that it eliminates the first pass effect influence and retains most of the physiological properties of the lung tissue; hence, it is much closer to the in vivo system in comparison to the in vitro lung cell model. However, IPL requires great skill and precision for removing the intact lungs from the animal. Other experimental challenges, like mounting the tissue and maintaining an experimental physiological condition, are of paramount importance for success in experimentation [51]. Another most convenient ex vivo model is precision cut lung slices (PCLS). PCLS can be prepared using murine models (rats and mice). For PCLS, the lungs are filled with a low-melting agarose solution. The agarose filled lungs are maintained in cold conditions to allow gelation, and the lungs are then sliced to the desired thickness using a tissue slicer. The entire slicing process is performed in cold cell culture medium, ensuring that the viability of the slices is maintained. The slices are then washed a minimum of three to four times using cell culture medium to remove traces of agarose. The slices can also be cut in specific positions, e.g., slices, including alveoli and pulmonary vessels. This way, the slices can be utilized for experiments with drugs involving the contraction and relaxation of the pulmonary vessels. In this way, the contraction-relaxation intensity can be observed by video microscopy, and special software calculations are performed using the area of contraction-relaxation of the vessel. The slices

being the actual lung tissue retain most of the physiological properties and receptor mechanisms, along with the inflammatory responses and are viable up to three days. Toxicity testing of sildenafil-loaded SLN and plain SLN was performed using PCLS as an ex vivo model [45,52,53].

5.1.3. In Vivo Models

In vivo models utilize whole animals to investigate drug absorption and disposition in the lungs. Commonly employed species include small rodents, such as mice, rats, and guinea pigs. However, mice pose challenges due to their small size, making blood sample collection and lung dosimetry difficult. Larger animals, including pigs, rabbits, sheep, and monkeys, are also used, particularly for studies focused on inhalation pharmacokinetics, formulation efficacy, and device performance. These models are more expensive but provide valuable insights. The most frequently employed method for administering drugs in these models is intratracheal instillation. Intratracheal instillation involves exposing the animal's trachea and making a small incision between the tracheal rings to insert an endotracheal tube. A microsyringe is then used to instill a precise volume (typically 10-200 μL) of an aqueous suspension or solution of the test formulation through the tube [47]. Alternatively, animals can be exposed to aerosolized formulations in a chamber, where the nose is secured, allowing for inhalation of the released aerosol (nose-only model). Following a predetermined exposure period, the animal is humanely euthanized, and the bronchoalveolar lavage fluid (BAL) is collected and analyzed for the desired components. Studies have been conducted to investigate the effects of Solid Lipid Nanoparticle (SLN) aerosol on lung function and toxicity. In these studies, mice were exposed to SLN aerosol for 16 days, and cytotoxicity was assessed through analysis of bronchoalveolar lavage (BAL) fluid, cytokines, and histopathological evaluation of lung tissue [3,4]. Intratracheal instillation requires expertise in tracheotomy surgery and involves collecting multiple blood samples to determine drug kinetics. In contrast, the nose-only model is less invasive and offers greater flexibility, with faster analysis of BAL fluid differential counts compared to multiple blood samples. Several experimental parameters must be considered when designing such studies, including animal selection, breathing frequency, device specifications, flow rate, inter-animal variations, and uptake mechanisms. Numerous in vivo models have been established to mimic various lung disease conditions, including: Monocrotaline sodium-induced pulmonary hypertension, Bacterial infection models for evaluating anti-tuberculosis formulations, Lipopolysaccharide (LPS)-induced

airway inflammation, Acrolein-induced airway inflammation and mucus production in murine models, These models are essential for in vivo testing and play a crucial role in preclinical studies [54-57]. The primary objective of these experiments is to determine the safe dosage range of a formulation, which is critical for progressing to Phase 1 clinical trials in humans.

VI. TOXICOLOGICAL ASSAY

In vitro and ex vivo toxicity testing encompasses a range of assays to evaluate the toxicity of drug formulations. These assays target various cellular components, including;- Mitochondrial function, Cell membrane integrity, Nuclear staining, Lysosomal activity, DNA ladder assays for assessing apoptosis and cell death mechanisms. These assays enable the assessment of potential toxic effects on different cellular structures and functions, providing a comprehensive understanding of the drug formulation's safety profile. Toxicity assays are a crucial component of the preclinical phase, providing invaluable insights into the safety profile of drug formulations and informing the selection of safe doses for in vivo animal studies. A comprehensive evaluation of data from in vitro, ex vivo, and in vivo testing, considering multiple experimental parameters, is essential to ensure accurate interpretation of results. To minimize errors and enhance statistical analysis, it is recommended to employ multiple assays in in vitro and ex vivo experiments. For instance, the MTT and resazurin assays, which assess mitochondrial activity, should be run in parallel to validate results and improve data reliability. Additionally, assays with distinct mechanisms can provide complementary information and should be performed simultaneously to gain a more comprehensive understanding of the drug's toxicity profile [58].

VII. Recent Applications

Due to their unique properties—a high surface area-to-volume ratio, variable size, and capacity to encapsulate a variety of drugs—nanoparticles have received a lot of attention in the field of pulmonary drug delivery. Nanoparticles are an appealing option for the controlled and targeted delivery of drugs to the lungs because of these properties. Recent advancements and applications in the field of nanoparticle-based pulmonary drug delivery include the following:

7.1.1 Treatment of Respiratory Diseases- For the treatment of a variety of respiratory conditions, including asthma, COPD, lung cancer, and pulmonary infections, extensive research has been conducted on nanoparticles. Targeted and sustained drug delivery to the lungs can be achieved by encapsulating therapeutic agents such as bronchodilators, anti-inflammatory drugs, antibiotics, or anticancer drugs within nanoparticles, enhancing therapeutic efficacy and decreasing adverse effects.

7.1.2 Targeted Delivery to Specific Lung Regions- Nanoparticles can be made to target particular parts of the lungs, like the alveoli or the deep lung, which are where many respiratory illnesses start. Enhanced drug delivery to the targeted sites is made possible by surface modifications of nanoparticles with ligands or antibodies specific to receptors or antigens. While minimizing systemic exposure, this strategy increases drug concentration at the desired site.

7.1.3 Inhalable Vaccines- As vaccine carriers for inhalable vaccines, nanoparticles have demonstrated promise. The immune response can be enhanced by encapsulating antigens in nanoparticles, enhancing vaccine efficacy. Advantages of inhalable nanoparticle-based vaccines include the possibility of self-administration, ease of storage and transportation, and the lack of the need for a needle.

7.1.4 Controlled Release Systems- Nanoparticle-based controlled release systems enable prolonged drug release and reduced dosing frequency. This strategy has the potential to reduce drug concentration swings and increase patient compliance. To achieve controlled drug release in the lungs, a number of methods, such as nanoparticle encapsulation, surface modification, and polymer matrix systems, have been developed.

7.1.5 Imaging and Diagnostics- Lung imaging and diagnostics can benefit from the use of nanoparticles as contrast agents. The precise diagnosis and monitoring of lung diseases is made possible by the surface functionalization of nanoparticles with specific imaging agents or targeting ligands.

7.1.6 Combination Therapy- By encapsulating multiple drugs with distinct mechanisms of action, nanoparticles provide a platform for combination therapy. Improved therapeutic outcomes, decreased drug resistance, and synergistic effects are all made possible by this strategy. For the treatment of lung infections, for instance, nanoparticles can be used to deliver both an antimicrobial and an anti-inflammatory drug simultaneously.

7.1.7 Nanoparticle Safety and Toxicity Studies- In addition to the development of pulmonary drug delivery systems based on nanoparticles, extensive research is being done on the safety and toxicity of nanoparticles. For their successful clinical translation, it is essential to comprehend the potential adverse effects and optimize the physicochemical properties of nanoparticles to minimize toxicity.

Despite the fact that nanoparticle-based pulmonary drug delivery holds a lot of promise, more research

is needed to address issues like large-scale production, regulatory approval, and long-term safety evaluation before widespread clinical use is possible.

VIII. CONCLUSION

In conclusion, a look at the most recent development in pulmonary drug delivery systems based on nanoparticles reveals significant advancements in this field. For targeted drug delivery to the lungs, nanoparticles have numerous advantages, including increased therapeutic efficacy, prolonged drug release, enhanced bioavailability, and decreased systemic toxicity. In order to overcome the difficulties associated with pulmonary drug delivery, a variety of nanoparticles formulations, such as liposomes, polymeric nanoparticles, solid lipid nanoparticles, and dendrimers, have demonstrated promising results. The optimal particle size, surface characteristics, and drug-loading capacity of these nanoparticles can be engineered to ensure effective drug release at the intended site of action and efficient lung deposition. The functionality and performance of nanoparticles-based pulmonary drug delivery systems have also been enhanced by the utilization of cutting-edge technologies like surface modification methods, formulations for inhalable aerosols, and nanocomposite systems. Asthma, chronic obstructive pulmonary disease (COPD), and lung cancer can now be treated with a wide range of therapeutic agents, including small molecules, peptides, proteins, and nucleic acids, thanks to these advancements.

However, despite the promising developments, there are still a few obstacles to overcome. These include maximizing the stability of nanoparticles, regulating the distribution of particle sizes, ensuring long-term safety, and overcoming potential respiratory clearance mechanisms. In addition, the successful transition of nanoparticle-based pulmonary drug delivery systems from research to clinical applications necessitates the standardization of manufacturing procedures, considerations pertaining to regulations, and scalability of production. In general, recent advancements in pulmonary drug delivery systems based on nanoparticles have demonstrated significant potential for enhancing the outcomes of lung disease treatment. To overcome the remaining obstacles and realize the full potential of these innovative drug delivery systems in clinical practice, scientists, clinicians, and regulatory authorities must continue to collaborate and conduct research.

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