CHAPTER - 2

REVIEW OF LITERATURE

2.1 ANATOMY AND PHYSIOLOGY OF THE RESPIRATORY SYSTEM

The respiratory system assists with the circulatory system to deliver oxygen from the lungs to the cells and carbon dioxide removal and return it to the lungs to be exhaled. The exchange of oxygen and carbon dioxide between the air, blood and body tissues is called as respiration. Healthy lungs take in about 1 pint of air about 12–15 times each minute. Whole of the blood in the body is passed through the lungs every minute. The respiratory tract is divided into two major parts: the upper respiratory tract, consisting of the nose, nasal cavity and the pharynx; and the lower respiratory tract consisting of the trachea, larynx, bronchi and the lungs (Figure-2.1).

![Figure 2.1: Different regions of the human respiratory tract](image)

The trachea, which start at the edge of the larynx, divides into two bronchi and continues into the lungs. The trachea allows air to move from the larynx to the bronchi and then to the lungs. The bronchi divide into smaller bronchioles which divide in the
lungs forming passageways for air. The terminal parts of the bronchi are the alveoli. The alveoli are the active units of the lungs and they constitute the site of gaseous exchange. (Gangurde et al., 2009).

The trachea is a continuation of larynx and extends downwards to about the level of the 5th thoracic vertebra where it bifurcates at the carina into the left and right bronchi, one bronchus going to each lung. It is approximately 10-11 cm long and lies in the median plain in front of the esophagus. The bronchi are composed of same tissue as the trachea. They are constituted with ciliated columnar epithelium. The bronchi progressively subdivide into bronchioles, alveolar ducts, terminal bronchioles and finally alveoli. Towards the distal end of the bronchi the cartilages become irregular in shape and are generally absent at bronchiolar level.

In the trachea bronchial region, a high proportion of the epithelial cells are ciliated in such a way that there is a approximately complete covering of the central airways by cilia. Towards the periphery at the tracheobronchial region, the cilia are least abundant and are absent in the alveolar region. Each ciliated cells have about 200 cilia with large number of interspersed microvilli, of about 1–2 μm in length. The cilia are similar to hair-like projections about 5 μm in length and 0.25 μm in diameter. They are submersed in an epithelial lining fluid, secreted through the serous cells present in the sub-mucosal glands. The tips of the cilia project through the epithelial lining fluid into a layer of mucus secreted usually from goblet cells. The cilia beat in an systematic fashion to propel mucus along the airways to the throat (Karhale et al., 2012).

Alveoli are small and there are approximately 300 million of them in each lung. Although alveoli are minute structures, they have a much large surface area in total (~100 m2) for performing precise gas exchange. The blood barrier between the alveolar space and the pulmonary capillaries is largely thin to allow for rapid gas exchange (Gangurde et al., 2009). Alveoli region is devoid of mucus and has a much flatter epithelium, which results into the simple squamous type, 0.1–0.5 μm thick. Two principal epithelial cell types are present:
• Type-I pneumocytes: Thin cells provide a very short airways-blood pathlength for the diffusion of drug and gases molecules. Type-I pneumocytes requires upto 93% of the surface area of the alveolar sacs, despite being half as abundant as type-II cells.
• Type-II pneumocytes: Cuboidal cells that secrete and store pulmonary surfactant.
Alveolar macrophages account up to ~ 3% of cells present in the alveolar region. These phagocytic cells transport and scavenge particulate matter to the mucociliary escalator and lymph nodes. (Karhale et al., 2012).

Alveolar macrophages are in contact with the alveolar epithelial cells and are responsible for the clearance of particles deposited in the alveolar region, in which mucociliary clearance is absent. They constitute the major non-inflammatory defense system in alveoli. In response to deposited nanoparticles, alveolar macrophages will migrate towards the nanoparticles and phagocytise them by chemotaxis consisting of opsonisation. Macrophage uptake is thought to complete in 6–12 h after particle deposition in the alveoli. Once internalised in the macrophages, the particles are either accumulated or disintegrated (e.g. by enzymes in lysosomes) in the lymphatic system, which drains the alveoli and airways. A small fraction of the particle carrying macrophages move to the ciliated airways, where they are eliminated by mucociliary clearance. The uptake of deposited particles by alveolar macrophages based on the composition and size of particles of any coating material. It has been shown that particles of 1–3 μm in diameter are taken up far better by macrophages, which have cell diameters of about 15–22 μm, than particles of 6 μm. Particles of less than 0.26 μm, on the other hand, can escape phagocytosis by macrophages (Bowden, 1984).

**Lung Surfactant**

Alveolar epithelium is covered with a thin liquid layer (< 0.1 μm) that has a surfactant film on its top. This surfactant is a complex mixture of proteins and lipids. Its main function is to decrease the surface tension at the alveolar air–liquid interface of lungs to prevent alveolar collapse at the end of expiration and facilitate the work of breathing. It is synthesized by type II pneumocytes and it follows a regulated exocytic pathway leading to secretion into the thin aqueous layer covering the alveoli. The composition of surfactant is 80-86% phospholipids, 6-12% proteins and 8% neutral lipids (mainly cholesterol). Saturated phosphatidylcholine accounts for 70% of the phospholipid portion of surfactant, with dipalmitoylphosphatidylcholine (DPPC) accounting for 60% of the phosphatidylcholine. DPPC is critical for decreasing the surface tension and can reduce it to almost zero. Although DPPC is the major component for surface activity,
alone it adsorbs poorly onto the air-liquid interfaces within the alveoli. The presence of unsaturated phospholipids aids the adsorption and surface active properties of surfactant (Akella and Deshpande, 2013; Weaver and Whitsett, 1991)

2.2 PHYSIOLOGICAL FACTORS AFFECTING PARTICLE DEPOSITION IN THE AIRWAYS (Karhale et al,2012)

2.2.1 Lung Morphology: Every successful manufacture of the tracheobronchial tree prepare airways of falling length and diameter. Each bifurcation produces an increase possibility for impaction and the decrease in diameter of airway is associated with a smaller displacement essential for a particle to provide contact with a surface.

2.2.2 Inspiratory Flow Rate: As the inspiratory flow rate increases they cause high deposition by impaction in the first few generations of the Tracheo-Bronchial region. The increase in flow, not only make particle momentum higher but also produces an increase in turbulence, mostly in the larynx and trachea, which itself will increase impaction in the proximal TB region.

2.2.3 Co-ordination of Aerosol Generation with Inspiration: The energy of aerosol particles developed from pressurized metered dose inhalers (p MDIs, is highly govern by the pMDI formulation instead of the subject’s IFR. pMDI aerosol droplets will be moving at velocities of 2,500–3,000 cm s−1. A failure to regulate actuation of the p-MDI during the early on phase of the inspiratory plan will result in near total particle impaction in the oropharyngeal area.

2.2.4 Tidal Volume: An increase in IFR will generally be connected with an enlarge in the volume of inhaled air in one breath, the tidal volume. Obviously an increase in tidal volume will produce penetration of aerosol particles deeper into the Tracheo-Bronchial and A regions and a better oppertunity for deposition inside these regions.

2.2.5 Breath Holding: Increasing the time between the end of inspiration and the initiation of exhalation enhance the time for sedimentation to occur. Breath-holding is generally used to optimize pulmonary drug delivery.

2.2.6 Disease States: Bronchial obstruction as watched in different pulmonary disorders may connected with the larger turbulence and local airflows and this will produce localized deposition in the wider airways of the trachea-bronchial region. The
bronchoconstriction of asthma has a much influence on exhalation than inhalation and thus deposition by sedimentation may be superior than normal.

2.3 PARAMETERS INFLUENCING LUNG DEPOSITION (Patton and Byron, 2007)

2.3.1 Patient features: The site and extent of lung deposition of particles depends on several patient-related factors. These are mainly the breathing pattern of the patient (i.e. flow rate, ventilation volume, and end-inspiratory breath-holding). Fast inhalation (high flow rate, Q) leads to increased deposition by impaction in the larynx. This significantly reduces penetration into the deep lungs, even for particles with diameters of about 2-3 μm. Moreover, the residence time of inhaled particles also based on the flow rate (slow flow rate = long residence time) and on the exhaled and inhaled volume (deep breath = longer residence time). A deep, slow breathing movement provides the inhaled particles much more time to deposit by diffusion and sedimentation, which increases deep lung delivery. This effect can also be obtained by implementation of an end-inspiratory breath-hold (5-10 seconds) in the breathing movement.

Another important factor affecting the lung deposition is the time of injection of the aerosol bolus into the inhaled volume. The first aerosol particles that enter the respiratory system penetrate deeper into the lungs than particles that are inhaled at the end of a breath. In summary, a slow, deep inspiration, with aerosol bolus administration at the beginning of the inspiration and a slightly delayed expiration, will have a positive impact on the quantity of drug delivered at the alveolar level. The optimum conditions include an inspiratory flow rate of 200-400 ml/s and an inhaled volume of 1000-2000 ml.

It is to be noted that the site of lung deposition, and particularly extrathoracic deposition, has high intersubject variability because of large anatomical and morphometrical differences in the mouth and throat, and variations in the diameter and branching patterns of the airways. Moreover, some diseases, such as asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and lung cancer, may cause changes in the pulmonary tract by obstruction due to excessive production of mucus or constriction of the airways (Scheuch et al., 2006).
2.3.2 Aerosol Features: The site and extent of lung deposition also depends strongly on the aerodynamic particle size distribution of the aerosol, which should be used in place of geometric diameters. The aerodynamic diameter of a particle is defined as the diameter of a sphere with a unit density that has the same settling velocity in still air as the particle in consideration. It depends on the geometric diameter of the particle (dgeo), its density (ρp), and the dynamic shape factor (χ), which is a dimensionless measure of the deviation from sphericity:

\[ d_{ae} = d_{geo} \sqrt{\left( \frac{\rho_p}{\rho_0 \chi} \right)} \]

Consequently, the aerodynamic diameter can be decreased by decreasing particle density, decreasing the particle size, and/or increasing the dynamic shape factor (Dolovich et al., 2005).

Figure 2.2: The effect of aerodynamic particle size on the deposition of aerosol particles in the human respiratory tract

According to the deposition mechanisms previously mentioned, drug-containing particles need to have aerodynamic diameters of between 1 and 5 μm to reach the lower respiratory tract and optimise pulmonary drug deposition (Figure-1.2). Indeed, particles larger than 5 μm usually impact in the oropharynx, from which they are easily cleared. In contrast, particles smaller than 1 μm may not deposit at all because of Brownian motion: they stay in suspension in air until exhaled. If systemic action is sought for the drug to be delivered, the target aerodynamic diameter must even be as low as 1-3 μm to reach the respiratory bronchioles and alveoli. Indeed, as an aerosol particle penetrates more deeply
into the lung, the airway epithelium becomes thinner and the lung surface area becomes larger, which increases the rate and extent of absorption (Ganderton, 1997).

The particle size distribution of a formulation is mostly characterized by its Mass Median Aerodynamic Diameter (MMAD), the Fine Particle Fraction (FPF) and the Fine Particle Dose (FPD). The FPD is the particles mass with an aerodynamic diameter below 5.0 μm, which are however expected to reach the lungs. The FPF is the fraction of the total drug dose with a particle size below 5.0 μm (Shah et al., 2012; Carvalhoa et al., 2011).

2.4 UPTAKE OF INHALED DRUG AFTER INHALATION THERAPY

There are many advantages in delivering drugs, to the lungs including a non-invasive method for delivery; the surface area of the lung is between 80 m² and 140 m². In addition, in most pulmonary regions, the thickness of the alveolar epithelium lies between 0.1 μm and 0.2 μm. The total distance between blood in the alveolar area and epithelial surface is between 0.5 μm and 1.0 μm which are very less than in the bronchial system (distance between blood and mucus surface: 30 μm–40 μm). Thus, it appears that pharmaceuticals after deep deposition and inhalation in the peripheral (i.e. alveolar) region of the lung can be rapidly absorbed. Pulmonary delivery however has the advantage, and compared to nasal delivery, that it is possible to obtain a sufficiently high absorption without the requirement of enhancers. Another advantage is that these drugs are not subject of a hepatic first pass effect after their absorption (Seville et al., 2008).

On the other hand, the human lung has different defense mechanisms to avoid aerosol particles penetrating into the deep lung. Primarily, the bronchial tree and the oropharyngeal region are excellent filters to remove aerosol particles from the inhaled air and particles deposited on ciliated epithelium are exposed to mucociliary transport to the gastrointestinal tract. However, to transport a drug into the deep lung, one has to overcome these filters. However, even after deposition in the alveolar region of the lung, a large number of mechanisms prevent the absorption of inhaled pharmaceuticals. There are a numerous absorption barriers (i.e. alveolar lining fluid layer, mucus layer, macrophages and other cells, basement membrane and alveolar epithelium) which act to different extents by preventing drug permeation into the circulation, there exists
competing cellular uptake pathways (e.g. particle phagocytosis by macrophages), and of course proteolytic degradation may decrease the amount of intact drug available for absorption (Pilcer and Amighi, 2010).

The function of these barriers can be destroyed by very different substances and consequently the absorption of drugs can be enhanced, for example, by the help of absorbance enhancers (e.g. detergents, cyclodextrins and bile acids). Furthermore, proteolytic degradation can be prevented by protease inhibitors (e.g. aprotinin and nafamostat mesilate) and phagocytosis by macrophages decreased by packaging of substances into porous particles. In principle, absorption kinetics of inhaled substances based on their molecular weight (small molecules are more fastly absorbed than larger ones), electrical charge, pH-value, stability and solubility of the inhaled substance. The other target regions within the lung for inhalable drugs are the small and large bronchial airways. Different pulmonary diseases are found in these parts of the respiratory tract.

The most relevant are: asthma, bronchial tumors and chronic obstructive pulmonary disease (COPD). To cure these diseases locally, one has to distribute the drugs specifically to this region. Therefore, a minor proportion of drugs can be absorbed into systemic circulation after such type of tracheobronchial deposition. In contrast to the inhalation of drugs for systemic cure, the inhalative therapy of COPD and asthma by means of metered dose inhalers (MDI) and nebulizers has been clinically established for many years and the treatments consist generally of low molecular weight molecules in formulations free of absorption enhancers and stabilizers. (Shah et al., 2012).

2.5 APPROACHES IN PULMONARY DRUG DELIVERY

Targeted drug delivery to the lungs has developed to be one of the much widely investigated local drug or systemic delivery approaches. The use of drug delivery systems (DDS) for the prevention of pulmonary diseases is enhancing because of their potential for localized topical therapy in the lungs. This route also makes it feasible to deposit drugs overall amount of drug given to patients (10–20 % of the per oral quantity), as well as enhancing local drug activity while decreasing first-pass metabolism and systemic side effects. (Gangurde et al., 2009; Baseir and Kellaway, 1998).
Particulate drug carriers such as microparticles, liposomes and nanoparticles can be used to improve the therapeutic index of established or new drugs by reducing metabolism, modifying drug absorption, prolonging biological half-life or reducing toxicity. Drug distribution is generally controlled primarily by properties of the carrier and no longer by physico-chemical features of the drug substance only. A careful design of such Drug Delivery System, depend on a thorough understanding of the clinical requirements for the disease conditions to be cured. Appropriate selection of the carrier materials, lung architecture / physiology, production process and device, are key to successful delivery (Chhayani et al., 2013; Courrier et al., 2002).

The biotechnology discoveries developed a wave of therapeutic proteins, also called as biomolecules, biotherapeutics, macromolecules, and biological. Many are administered via intravenous methods or injection to avoid destruction in the gastrointestinal tract. Patients, however, fear and avoid injections and Intravenous treatments, which are inconvenient, painful, and expensive. Pulmonary delivery provides a patient-friendly, non-invasive alternative to injections and can also be a more precise and effective way to deliver a drug and develop patient compliance (Gangurde et al., 2009).

2.5.1 Microparticles: The terminology “microparticle” (size comprised between 1 and 999 μm) includes the microcapsules and the microspheres (uniform sphere constituted of a polymeric matrix). Biodegradable microspheres, designed from synthetic or natural polymers, have been largely used as drug targeting systems via different routes. Lipophilic and hydrophilic molecules can be incorporated or encapsulated into microspheres. Compared to microspheres or liposomes have an in vitro and in vivo more stable physicochemical behavior and should permit a slower release and a extensive pharmacological activity of the encapsulated drugs. Biodegradable microspheres are prepared by using different polymers: chitosan, albumin, polysaccharide, poly (lactic-glycolic) acid, poly (butylcyanoacrylate), poly (lactic) acid and poly (lactic-co- lysine graft lysine).

Pulmonary administration of aerosolized microspheres allows a sustained and prolonged release of drugs for respiratory or non respiratory diseases, in this last case, the drug being protected against the enzymatic hydrolysis. Microspheres can be produced
following different requirements such as the size, the morphology and the porosity by varying different technological parameters during their preparation. Microspheres are least hygroscopic and are less liable to swell in the presence of moisture present into the lungs (Chhayani et al., 2013; Dolovich et al., 2005).

(i) Sustained Release Microparticles: Till now sustained release formulations for pulmonary delivery have still not been marketed instead of the enhancing interest in this research field. The control of the drug delivery in the respiratory tract may be affordable by providing suitable carriers, possessing appropriate drug release features. In this purpose, liposomes have been the mainly studied carriers. They proved to be able to provide a sustained release to the incorporated medicaments but they exhibit some disadvantages, i.e a relative instability during storage, a high production cost and during nebulisation that can led to loss and disruption of entrapped substance (Robertson et al., 1998).

Polymeric microspheres have been successfully examined as sustained release drug delivery system but their safety still remains doubtful. Due to this reason, Solid Lipid Microparticles (SLMs), a carrier that has not been till now much studied especially for pulmonary administration. Therefore, SLMs present several advantages: they can be considered as physicochemical stable, physiologically compatible and allowing a large-scale production at a relative low production cost. The objective of the work was to produce a drug carrier able to provide a sustained release to a β2-mimetic agent and thereby to prolong its duration of action. The active substance such as salbutamol acetonide (SA) was choosen as a derivative of salbutamol that have been synthesized in order to get a more lipophilic substance and thereby to allow a much effective incorporation of this drug into SLMs (Gangurde et al., 2009).

2.5.2 Nanoparticles: Nanoparticles exhibit the same characteristics than the microspheres, they are also consist of polymers or lipids and drugs bound either at the surface of the particles or encapsulated into the vector. In this last case, a protection against the enzymatic degradation and a modified bioavailability of the drug can be determined and enhanced by a controlled release (Leversha et al., 2000). These targeting systems can be developed for in vivo applications contain molecules with therapeutic activities and radio contrast agents or in vitro as a support for molecules intended for
diagnosis. Manufacturing and encapsulating methods for drugs and the feasibility of modifying the surfaces of these vectors have been reviewed by different authors. Drug targeting studies using these vectors by pulmonary route have been essentially conducted by encapsulating insulin (Chhayani et al., 2013).

(i) Sustained Release Nanoparticles: A pulmonary drug delivery system to prevent tuberculosis provides a number of advantages over current oral medications. By delivering antibiotics via inhalation, the infected tissues of the lung are directly targeted while maintaining lower systemic drug toxicity and concentrations. Preliminary studies on pulmonary delivery of para-amino salicylic acid (PAS) in rats have exhibited this method to indeed allow for minimal inhibitory drug concentrations (MIC) to be present in lung tissue with more lesser systemic tissue drug concentrations. In addition, previous studies on the application of polymeric nanoparticles for drug delivery have exhibited that it is possible to encapsulate and deliver a range of drug molecules and proteins (Dewar et al., 1999). Polymeric and lipid nanoparticles shells are promising candidates for this delivery method because of low density and their large size, which causes them to deposit in the alveolar region (where there is good contact with the bloodstream) and prevent removal from the lungs. In addition, the porous shell surface permits for the slow, sustained release of TB drugs, which may interpret into attenuated and a less frequent drug treatment regimen (Dewar et al., 1999).

2.5.3 Micelles: A successful drug carrier system requires to demonstrate optimal drug loading and release properties, low toxicity and long shelf-life. Colloidal systems, such as vesicle and liquid crystal dispersions, micellar solutions, as well as nanoparticles dispersions consisting of small particles of 10–400 nm diameter exhibit great promise as carriers in pulmonary drug delivery systems. Drugs may be trapped in the core of a micelle and transported at concentrations even larger than their intrinsic water solubility (Gazarian et al., 2001). A hydrophilic shell may form around the micelle, effectively protecting the contents. In addition, the outer chemistry of the shell may avoid recognition by the reticuloendothelial system, and however early removal from the bloodstream. A further feature that makes micelles attractive is that their shape and size can be changed. Chemical techniques using cross linking molecules may improve the stability of the micelles and their temporal control. Micelles can also be chemically
altered to selectively target a wide range of disease sites (Karhale et al., 2012; Thulasiramaraju et al., 2013).

2.5.4 Liposomes: The utilization of formulations of liposomal drug for aerosol delivery has many potential benefits, including sustained pulmonary release to maintain therapeutic drug levels, aqueous compatibility and facilitated intra-cellular delivery particularly to alveolar macrophages. Moreover, drug-liposomes may avoid local irritation and reduce toxicity both systematically and locally. Enhanced potency with reduced toxicity is characteristic of many drug-liposomal formulations (Barry and Callaghan, 2003).

Liposomal aerosols (including CsA) have found to be non-toxic in animal and acute human studies. These results suggest that drug-liposome aerosols should be much effective for deposition, delivery and retention of hydrophobic, water-insoluble, lipophilic compounds in contrast to water soluble compounds. The preparation of liposomal formulations for aerosol delivery with jet nebulizers has enlarged the possibilities for effective utilization of aerosol dependent therapies in the cure of pulmonary diseases. The property of sustained release or depot effect of liposomes has been studied using various tracer molecules to monitor absorption and clearance of liposomes from the lung (Dolovich et al., 2005).

The preparation of liposomal formulations, compatible with aerosol delivery with jet nebulizers, has also enlarged the possibilities for much effective utilization of aerosol dependent therapies for the prevention of a variety of pulmonary diseases. Such utilization of liposomes, as aerosol delivery vehicles, has much reported potential benefits for clinical development, including: aqueous compatibility facilitated intra-cellular delivery particularly to lymphocytes and alveolar macrophages and sustained pulmonary release to stabilize therapeutic drug levels within the lung (Karhale et al., 2012; Thulasiramaraju et al., 2013).

2.5.5 Microemulsions: The microemulsions and emulsions are dosage forms showing large number of advantages providing that the surfactants used are not toxic. Anyway, more and more exogenous surfactants, used for preventions and as a precaution for acute respiratory distress syndrome (ARDS), are used as suspensions or solutions, drug targeting systems. Therefore, these permit envisaging at once a respiratory treatment and
a drug delivery system (Rau, 2005). These surfactants are considered as efficient drug targeting systems if they do not interfere with the therapeutic activity of the drug. Very few microemulsions or emulsions have been examined to administer drugs by the pulmonary route. Therefore, these dosage forms exhibit numerous benefits compared to other drug targeting systems: maximum of drug to be incorporated and easiness to be manufactured (O’connor, 2004). Indeed, the drug being soluble into one phase, this one will be present preferentially into this phase, causing an encapsulation close to 100%. Due to their physicochemical features, reverse microemulsions and emulsions should permit to solubilize a high amount and a lot of hydrophilic drugs (Gangurde et al., 2009; Rau, 2005).

Several aerosol formulations developed with an external phase constituted of a propellant have been explained. Propellants like propane or hydrofluoroalkanes (HFAs) have been suggested. Reverse microemulsions stabilized by lecithin and using dimethylether and propane as propellants have been also discussed (Newman and Clarke, 1993). These microemulsions, evaluated by mean geometric diameters ranged between 1 and 5 μm and by a respirable fraction up to 36%, exhibited high stability during more than 4 weeks at room temperature. Water-in-HFA emulsions stabilized by non ionic fluorinated surfactants have been examined in order to administer drugs by pulmonary route and studied new reverse microemulsions and miniemulsions dependent on fluorinated surfactants required for pulmonary delivery of drugs (Karhale et al., 2012; Gangurde et al., 2009; Thulasiramaraju et al., 2013).

2.5.6 Cyclodextrins: Cyclodextrins (CDs) are the due to the association of oligosaccharides and are prepared of six, seven or eight units of glucopyranose (α-, β- or γ-CD, respectively). Following the partial or complete inclusion of the drug into the cavity, the drug may interact by non covalent bonding with Cyclodextrins, becoming largely soluble in an aqueous medium. β-CD seems to be the much used cyclodextrins for pharmaceutical development, as a result of the size of its cavity, the complexation efficiency with drugs and their comparatively low production costs. CDs have been examined to encapsulate drugs and to be used in this application to target drugs into the lungs. Cyclodextrins are able to complex salbutamol, testosterone or rolipram. Cyclodextrins may be used also in combination with other vectors. They are able to
enhance the encapsulation rate of drugs into microparticles and to alter their releases. Principally, they were explained and used by pulmonary route for their pulmonary absorption promoter of proteins like insulin or calcitonin and peptides (Karhale et al., 2012; Ganderton, 1997).

2.6 APPLICATIONS OF PULMONARY DRUG DELIVERY

2.6.1 Applications of Pulmonary Drug Delivery in Asthma and COPD: Asthma is a chronic long term lung disease that is categorized by narrowing of airways and inflammation. Asthma causes repeated periods of chest tightness, wheezing, coughing and shortness of breath. The coughing often occurs at early in the morning or night. Asthma affects people of all ages, but it most frequently starts in childhood. COPD known as chronic obstructive pulmonary diseases, which is correlated to chronic bronchitis, smoking and emphysema. Today’s inhaled drug delivery market is conquered by the three main classes of drug such as corticosteroids, bronchodilators and anticholinergic. All these three classes of drugs are administered by pulmonary route only. levosalbutamol inhalers are located in the market to cure asthma. Titropium inhalers are located in market to treat COPD (Sheikh, 2011; Patton, 2006).

2.6.2 Applications of Pulmonary Delivery In Diabetes: Diabetes is a syndrome of disordered metabolism and unfortunate hyperglycemia obtained from a insufficiency of insulin resistance or secretion. Diabetes can results in a stroke, heart attack, blindness, nerve damage, kidney disease and other serious health problems. The most frequent form of this therapy is twice-daily subcutaneous insulin injections. This type of treatment is painful and as a result encourages rebelliousness by up to half of the diabetics. Proteins and/or peptides are becoming very important in medication. When these are taken orally, they are destroyed by the proteolytic enzymes in the gastrointestinal tract, and might be impervious to the intestinal mucosa due to their large molecular size and hydrophilicity. As a result, systemic administration of these macromolecular drugs and diagnostic agents and other therapeutic has been restricted to the parenteral route. Repeated injections are essential due to the short half-lives of peptide/protein drugs. The first attempts at intrapulmonary delivery were made in the 1920s. Several companies are making efforts on insulin inhalers than any other insulin delivery option (Huang and Wang, 2006).
2.6.3 Applications of Pulmonary Delivery In Angina Pectoris: Angina pectoris is indicated as symptoms of myocardial ischemia and it starts as a result of imbalance between demand of myocardium and oxygen supply. Nitroglycerine is a drug used for angina pectoris, and is administered through sublingual route. It is a coronary vasodilator. The fast relief of angina is probably provided by the subsequent reduced cardiac work and a reduced demand of oxygen on the heart. An aerosol form has been examined in Europe and has been observed comparable to sublingual nitroglycerine. In particular, its efficacy has been indicated better than nitroglycerine tablets in patients with dry mouth. Isosorbide aerosol has also been observed of use in hypertensive emergency (Karhale et al., 2012).

2.6.4 Applications of Pulmonary Delivery In Pulmonary Arterial Hypertension: Pulmonary hypertension in the setting of chronic hypoxia due to underlying lung disease shown a challenging area for management and evaluation. However chronic hypoxia is a well known cause of pulmonary hypertension, it would rarely lead to severe pulmonary hypertension. In 2004, the FDA approved Ventavis (iloprost), an inhaled cure for pulmonary arterial hypertension, made by CoTherix (South San Francisco, CA, U. S. A.). In pulmonary arterial hypertension, severe restriction of blood vessels lead to early death. Iloprost naturally expand blood vessels (Kumar et al., 2013; Robyn and Barst, 1998).

2.6.5 Application of Pulmonary Drug Delivery In Cancer Chemotherapy: Cancer is one of main disease which lead to death of people. Lung cancer is the leading reason of cancer deaths globally, and inhaled chemotherapy observed a logical approach to cure lung cancer. A multicenter Phase I clinical trial is characterizing doxorubicin HCl inhalation solution in lung cancer patients. As many as 400 000 lung cancer patients could take advantage from inhaled chemotherapy; a research is going on aerosolized paclitaxel solution to mice with lung tumors. The treatment effectively reduced prolonged survival and lung tumors. Aerosol delivery of the anticancer agent’s 5-fluorouracil and difluoro methylornithine reduced lung tumours in mice 60 % and 50 %, respectively. Interleukin-2 activate immune function in cancer patients, but injections cause malaise, fever, and local swelling (Chhayani et al., 2013).

2.6.6 Inhaled Drug Delivery For Tuberculosis Therapy: One third of the world population is suffering with tuberculosis (TB), and new infections occur at a rate of one
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per second. The recent enhance in the emergence of drug-resistant strains of *Mycobacterium tuberculosis* and the use of anti-TB drugs is scaring the future containment of TB. Delivery systems or new drugs that will stop the spread of TB and prevent or slow down the development of drug-resistant strains are urgently needed. One of the reasons for the development of drug-resistant strains is the exposure of mycobacteria to sub-therapeutic levels of one or more antibiotics. Lung lesions consisting of large numbers of bacteria are fortified with thick fibrous tissue and are poorly vascularized; conventional therapy by the parenteral and oral routes may provide subtherapeutic levels of anti-TB drugs to these largely sequestered organisms. Administering of drugs by the pulmonary route to the lungs permits higher drug concentrations in the gap of these lesions. Supplementing conventional therapy with inhaled anti-TB therapy may permit therapeutic concentrations of drug to penetrate precisely into lung lesions and cure the resident mycobacteria (Muttil et al., 2009).

2.6.7 Applications of Pulmonary Delivery For Bone Disorders: Disease such as Paget’s disease and osteoporosis of bones can be cured by pulmonary delivery. The estimated enhance in the number of patients with osteoporosis and the lack of ideal therapies indicate the need for better treatments. Clinical evidence from a variety of other proteins and peptides indicates that pulmonary delivery is efficient, safe, well preferred and tolerated by patients so pulmonary route is best option to cure bone disorders. Generally occurring peptides parathyroid hormone and calcitonin, used to prevent osteoporosis by regulating bone metabolism. For the peptides to become effective therapies, formulations must be prepared that bypass the need for injection. Pulmonary delivery of parathyroid and calcitonin hormone appears likely in the coming future. The recent administration of a nasal formulation of calcitonin points to the compatibility of lung delivery. A pulmonary formulation inhaled through the mouth that delivers PTH or calcitonin into the deep lung should improve the efficacy and bioavailability of the drugs (John, 2000).

2.6.8 Gene Therapy Via Pulmonary Route: Gene therapy holds high potential for the prevention of various inherited and acquired pulmonary diseases. Major aim of Gene therapy given gene transfer may significantly stimulate the underlying chloride defect in the lungs of patients with CFC. There are numerous problems to be overcome before
clinical uses are practical. Some of these are adequate gene expression, successful transfer of sufficient genetic material to appropriate tissue, safety, maintenance of expression over time, and efficacy of expression (Kumar et al., 2013; Kaur and Harikumar, 2013) (Figure-1.3).

Figure 2.3 : Nanoparticles for gene delivery

2.6.9 Recent use of Pulmonary Drug Delivery In Transplantation: Inhalation route play a very essential role in transplantation. During lung transplantation, an intrapulmonary shunt and pulmonary vascular pressure have been exhibited to respond to inhaled aerosolized prostacyclin and inhaled nitric oxide. Prostacyclin which is administered by pulmonary route has also been explained as an alternative to nitric oxide in the maintenance of reperfusion injury after lung transplantation. Chronic and acute rejections are main problems compromising patient survival and transplant. Aerosolized cyclosporine is beneficial for reducing the risk of acute rejection (Gabrielle et al., 2009).

2.6.10 Pulmonary Delivery In Cystic Fibrosis: Nowadays cystic fibrosis is very frequent disease. Pulmonary delivery illustrated an essential role in the prevention of cystic fibrosis for decades. The major aim of aerosol system is to transport drugs to children’s and infants. The following drugs are administered by pulmonary route for management of cystic fibrosis (Karhale et al., 2012).

1) N-Acetylcysteine: The mucolytic agents N-acetylcysteine (NAC) have been needed by pulmonary route to assist in sputum clearance. It will support to liquefy tenacious secretions and make their clearance easier. Recently newer mucolytic agent, nacystelyn, has been designed for delivery via a dry powder inhaler.
II) **Recombinant Human Deoxyribonuclease Aerosol**: Now a days deoxyribonuclease is administered by pulmonary route. Recombinant human deoxyribonuclease aerosol may be applicable to liquefy secretions in cystic fibrosis patient.

III) **Tobramycin-spray dried**: Tobramycin powders consisting Nanoparticles for pulmonary delivery. Tobramycin is frequently used to cure patients with cystic fibrosis. Overall, evidence predicts probably reduced hospitalization and improved lung function when tobramycin is part of maintenance therapy in cystic fibrosis.

**2.6.11 Role of Pulmonary Delivery In Vaccination**: When there was mild interest in aerosol vaccination 15–20 years ago, progress toward use has been modest seen. Nearly 100 vaccines are certified in the U. S. About half of these treat respiratory infections, yet all are currently injected. Recently, inhaled measles vaccine administered by nebulizer. As far back as the 1960, influenza experts examined aerosol flu vaccine (Kumar et al., 2013; Al-Tabakha and Alerid, 2008).

**2.6.12 In Emphysema**: Emphysema is mainly popular respiratory disease due to deficiency of Alpha 1 antitrypsin uncontrolled neutrophil elastase deficiency produced which leads to the formation of emphysema and lung destruction. Now a days recombinant AAT (rAAT) administered intravenously (IV) is very well accepted cure. Early testing of aerosolized AAT documented appropriate alveolar fluid AAT and penetration into the lung interstitum., Secretory leukocyte protease inhibitor and neutrophil elastics’s inhibitor have also been considered for protection against elastase in cystic fibrosis and patients with AAT deficiency (Karhale et al., 2012).

**2.6.13 Nicotine Aerosol For Smoking Cessation**: As smoking is injurious to health. It is much difficult to aces such habit. From ancient times, people smiles cigarette and get addicted with smoking. The main reason for cigarette smoking is nicotine addiction, and nicotine replacement is appealing as a means of decreasing cigarette use to ultimately obtain cessation (Karhale et al., 2012).

**2.6.14 Pulmonary Delivery Of Lower Molecular Weight Heparin**: Now days, low molecular weight the parins is best as an alternative to un fractionated heparin because of improved pharmacokinetic profiles and decreased cost of therapy in the cure of pulmonary embolism and deep vein thrombosis . Low molecular weight heparins are administered by intravenous and subcutaneous routes. Administration of an anticoagulant
drug directly to the pulmonary circulation should be ideal for the prevention of pulmonary embolism. A pulmonary formulation of LMWH will permit direct administration of the drug into the lungs, and as a result, this formulation is likely to decrease the mortality from an attack of pulmonary embolism. This pulmonary therapy is noninvasive because it is generally needle free (Kaur and Harikumar, 2013; Tianzhi et al., 2009).

2.6.15 Diagnostic Application Pulmonary Drug Delivery: Pulmonary drug delivery is not only used for therapeutic objective but also for diagnostic objective. For example, inhalation of aerosols of histamine and methacholine is responsiveness in asthma (Pavan et al., 2009).

2.7 CAPABILITY OF NANOPARTICLES TO TARGET LUNG TISSUES
2.7.1 Mechanism of Deposition of Nanoparticles in the Respiratory Tract: Size particularly is an essential determinant of whether or not nanoparticles will be precisely deposited deep into the lungs or if they will generally be exhaled. The sizes of particles which are employed for inhalation therapy are commonly expressed in terms of the mass median aerodynamic diameter (MMAD). The aerodynamic diameter is generally called as the diameter of a sphere of unit density, which have the same velocity in the air stream as a non-spherical particle of arbitrary density. This diameter generally interpret the mechanism of particle deposition in the respiratory tract (Martonen and Katz, 1993; Swift, 1980).

In general, aerosol particle size is assumed to be the mass median aerodynamic diameter (MMAD). The MMAD is used to describe the particle size distribution of any aerosol statistically depend on the size and weight of the particles. Hence, a group of very dense particles will show a larger MMAD than that of a group of less dense particles, although they have an identical geometric size. The technique for successful deposition needs that the particles must be small enough to prevent deposition by impaction in the upper respiratory tract and allow them to pass through the mouth, larynx, pharynx, and lower airways while simultaneously being large enough to avoid exhalation (Groneberg et al., 2003; Hickey and Martonen, 1993; Ferron, 1994).
As a result, density and particle size reflected in the MMAD of a particle are essential characteristics for lung delivery. The diameter of nanoparticle ranging from 30-50 nm and 1-3 μm have found to show high deposition in pulmonary region. Different studies have exhibited that diameters of particles ranging from 100–500 nm might be successfully deposited into different regions within the respiratory tract, when they are incorporated into appropriate vehicles such as dry powders or aerosols (Mansour et al., 2009).

### 2.7.2 Interaction of Nanoparticles with Lung Epithelia and Macrophages:

Based upon the location of deposition, the nanoparticles may interact with specific cell population within the lungs (Figure–1.4). Type I and II pneumocytes as well as ciliated epithelial cells are the primary cells within the deep respiratory tract that interact with receptor-mediated endocytosis is the mechanism mainly responsible for the intracellular nanoparticles uptake whereby opsonins (such as glycoproteins, proteins, and glycolipids) precipitate on the particle surface making a complex recognizable by receptors of macrophages which then can bind to the complex with their cell surface permitting for particle uptake through pseudopod extensions. Phagosomes then bind with lysosomes (containing acid hydrolyases) which have the property to destroy the drug delivery vehicle. During this process, therefore, the drug itself as well as the particle can be degraded by the action of enzymes and hence, the nanoparticles must first emigrate the lysosomes in order to retain activity. Nanoparticle delivery to the alveolar region permits drug targeting to the alveolar macrophage population and has essential magnitude for curing diseases which are caused by these immune cells. Moreover, nanoparticles have potential to be applicable for delivery of DNA and antigens and may be essential for vaccine delivery through the respiratory route (Bharti et al., 2013).

Macrophages are central in defending the lungs against the attacks of pathogens and particles in inspired air. Particles are not only ingested but undergo slow dissolution within the phagolysosomes of macrophages. The microbicidal and phagocytic potential of macrophages is one of the main reasons to keep lungs remain sterile and clean. Macrophages may also inhibit allergy by catabolizing and ingesting 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 increase the immune response (Brain, 1988).

Lung macrophages destroy and recognize neoplastic cells, thus inhibiting the development of cancer. Macrophages can secrete such different substances as interferon, lysosomal enzymes, angiogenesis factor, components of complement, cyclic nucleotides, plasminogen activator, prostaglandins, leukotrienes, granulopoietins and inflammatory cytokines. Diverse agents such as silica, viruses, ethanol intoxication, immunosuppressives, air pollution, cigarette smoke, hyperoxia and hypoxia can depress

\[\text{Figure 2.4: Interaction of nanoparticles with lung epithelia and macrophages}\]

ability of pulmonary macrophages to protect their host. There are also instances in which pulmonary macrophages not only fail but are themselves involved in the pathogenesis of pulmonary diseases. For example, the ingestion of particles (e.g., cigarette smoke), endotoxin or microbes, causes the release of oxygen radicals and lysosomal enzymes
into the external environment or the macrophage cytoplasm. These substances can damage other macrophages and surrounding cells; then dying or dead macrophages release substances that may elicit fibrogenic responses and attract fibroblasts. This extracellular release of oxygen radicals and proteases can also change the activity of a variety of enzymes or the extracellular matrix, then macrophages can be centrally employed in the development of lung disease (Brain, 1992).

**2.7.3 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 surfactant – lining fluid layer within the alveolar region or the airways (Figure-5). Airway mucous (about 5 μm in depth) is a complex aqueous secretion of airways, consisting of proteins, electrolytes, debris of cells and glycoproteins (mucins). The components differ much based upon disease states and environment. The surfactant lining layer (10-20 nm in thickness) that covers the alveolar surface is composed of 10% in weight of specific proteins and 90% in weight of phospholipids. Both alveolar surface liquids and airways are coated with at least a monolayer of highly surface active lung surfactant, which are mainly water insoluble long –chain phospholipids. They form liquid crystals but not micelles in aqueous media to maintain the functions of the lungs such as prevention of alveoli collapse by reducing the lung air interface surface tension and facilitation of gas exchange (Bharti et al., 2013).

An analysis of the interaction between nanoparticles and pulmonary surfactant is very much essential as dire consequences would result if such drug delivery systems destabilize the surfactant film coating the alveoli. Accordingly numerous surfactant film studies are carried out using a Langmuir Blodgett trough which allows one to mimic the physiological situation located within the respiratory tract (Yu and Possmayer, 2003). A recent study by Stuart et al., investigated the interaction of different nanoparticles with the lung surfactant film (Bharti et al., 2013) (Figure-1.5). Accordingly, the interaction between nanoparticles and dipalmitoylphosphatidylcholine (DPPC) a major component of native pulmonary surfactant was investigated (Bharti et al., 2013). A DPPC lipid monolayer was needed to simulate the surfactant layer of the respiratory tract (Park et al., 2005). The aim of this study was to investigate if the
deposition of nanoparticles in the alveolar region will compromise the strength of the surfactant film. Such interactions can cause dosage form associated incompatibilities and are essential for the preclinical characterization of the feasibility of nanoparticulate delivery of nanomedical devices. The incorporation of the particles into the lipid film was size dependent and had a measurable influence on the surface tension of the lipid layer. Therefore, the study also exhibited that nanoparticles do not significantly destabilize the surfactant film.

The method outlined in this study can be a suitable test to set limits to evaluate dosage form related nanotoxicological properties and for nanoparticle deposition of inhalable nanoscaled drug delivery systems. In another study, nanoparticles consist of D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) as well as other biodegradable substances as carrier matrix were investigated. TPGS is also investigated to stabilize pulmonary surfactant thereby allow its use in pulmonary drug delivery much attractive.
The study also pointed out that the TPGS coated nanoparticles do not destabilize the model surfactant film and have large potential for pulmonary drug delivery (Brain, 1992).

2.7.4 Retention and Clearance Mechanisms of Nanoparticles Deposited within the Respiratory Tract:

2.7.4.1 Deposition of Nanoparticles through the Respiratory Tract

The structure of the lung tissue largely differs according to airway generation and the fate of medicines will similarly differ based on the structures in which they deposit. The site of deposition of an inhaled formulations within the respiratory tract are based on the aerodynamic diameter (daer) of the particles of aerosols. Filtering of large particles (daer >5 μm) carried out in upper airways (mouth, main bronchi and trachea) by inertial impaction. One to 5 μm daer particles deposit by gravitational settling in the central and distal tract. Particles with daer <1 μm remain suspended in the air and are mainly exhaled. Ultrafine particles (<100 nm) may greatly deposit in the respiratory tract by random Brownian motion: particles <100 nm reach the alveolar region while particles <10 nm already deposit in the TB region due to their high diffusion coefficients (Bharti et al., 2013).

2.7.4.2 Clearance mechanisms of nanoparticles through the respiratory tract

Different removal pathways for nanoparticles present in the lungs, including dissolution, coughing, translocation from the airways to other sites, mucociliarily escalator, phagocytosis by macrophages and neuronal uptake (Figure 1.6); but the quantitative relationship among these pathways has not yet been developed. When a nanoparticle has arrive in the airways, it first encounters the surfactant on the top of the airway lining fluid. The surfactant will increase particle wetting hence assisting it to sink into the fluid, passing first through the gel phase and then to the sol phase. Compared with sulphur colloidal particles (220 nm), human serum albumin molecules (HSA, 66 kDa) were cleared 3 times more gradually from the bronchi of dogs. This difference was attributed to the possibility that sulphur nanoparticles present on the gel phase (i.e. the top layer of the periciliary fluid) whereas Human Serum Albumin dissolved and partitioned into the sol (i.e. bottom layer) which may be transported less precisely by mucociliary clearance (MCC). If extrapolated to inhaled drugs, the much soluble ones will behave like HSA and should be therefore less susceptible to MCC, but more likely absorbed
through the epithelium. For nanoparticle agglomerates, it is likely that the particles will first lie on the gel phase.

![Diagram of pathways involved in nanocarriers absorptions](image)

**Figure 2.6 : Pathways involved in nanocarriers absorptions**

Based on the solubility and aqueous dispersibility in the airway fluid, the agglomerates can remain in the gel phase behaving like the microsized particles, or they can then disperse into nanoparticles followed by absorption and dissolution. Nanoparticles delivered within a liquid droplet (e.g. from a nebulizer) could be different from dry particles, as the droplet liquid can interact with the gel layer developing the nanoparticles easier to partition and wet into the sol layer, i.e. potentially much readily to escape the MCC (Yu and Possmayer, 2003).

### 2.7.4.2.1 Dissolution

Dissolution based on the site of deposition, which find out the volume of airway fluids available for dissolution and hence, whether the dissolution takes place in non-sink or sink conditions, as well as on dose of the drugs and the solubility. Freely water soluble drugs consist of polar compounds (e.g. mannitol) and organic salts (e.g., terbutaline sulphate, salbutamol sulphate and disodium cromoglycate). These drugs will dissolve rapidly in the airway fluid followed by elimination or absorption by the mucociliary
escalator. Sparingly soluble drugs consist of the inhaled corticosteroids, which have aqueous solubility ranging from 140 to below 0.1 μg/mL. Once dissolved, the drug molecules are diluted in the airway fluid where they may bind to opsonins, proteins, or other constituents and be metabolized or absorbed into the lymph and blood (Groneberg et al., 2003).

Absorption of the drugs based on the site such as conducting airways or alveolar (which affects the surface area and barrier thickness) and the drug molecule itself (which influence on active uptake and passive diffusion by the epithelium. It must be concluded that absorption of many drugs from the lungs is fast: as an example, it has been reported that following inhalation of budesonide (136 pmol/L) and formoterol fumarate (4.5 nmol/L), the peak plasma concentrations occurred at 10 min and 20 min, respectively.

### 2.7.4.2.2 Mucociliary Clearance (MCC)

Mucociliary clearance operates in the ciliated airways where the movement of the cilia deliver the mucus carrying the dissolved drug (not yet absorbed) or drug nanoparticles on the epithelial surface towards the larynx/pharynx. The drug-consisting mucus will then be swallowed to the gastrointestinal tract. The average transport velocity in the human trachea has been estimated at 3–10 mm/min, but the value differ widely among subjects. Using well-developed methods of depositing radiolabelled sulfur colloids in the central airways, Daviskas and her colleagues reported a Mucociliary clearance rate remarkably decreased in patients with asthma, bronchiectasis, and cystic fibrosis, Novel Concept in Pulmonary Delivery 307 with respect to healthy individuals. Actually, dissolution and Mucociliary clearance occur simultaneously and their relative importance should based on the elimination rate from each of these contributions. While insoluble particles of 6 μm are practically all cleared from the bronchial airways by Mucociliary clearance in 24 h, smaller particles are retained for a longer period showing actually an inverse relationship between the geometric particle size and the 24 h airway retention. Nanoparticles with increased mobility can partition through the mucus into the periciliary spaces, where they may be taken up by bronchial epithelial cells and the airway macrophages, causing a reduction of Mucociliary clearance (Gangurde et al., 2009; Brain, 1992).
2.7.4.2.3 Macrophage Uptake

Alveolar macrophages are responsible for clearance of nanoparticles deposited in the alveolar region, in which mucociliary clearance is absent. In response to the deposited nanoparticles, alveolar macrophages will move towards the particles and phagocytize them via chemotaxis consisting of opsonisation. Macrophage uptake is determined to complete within 6–12 h after deposition of the particles in the alveoli. Therefore, internalized in the macrophages, the particles will be either accumulated or disintegrated (e.g. by enzymes in lysosomes) in the lymphatic system draining both alveoli and airways and at last terminating in the hilar and mediastinal lymph nodes (Brain, 1984).

A minute fraction of the particle-carrying macrophages will move towards the ciliated airways where they are eliminated by mucociliary clearance. Therefore, with a retention half-time of up to 700 days in humans, clearance of solid particles by alveolar macrophages is a comparatively gradual mechanism. Phagocytosis of particles below 100 nm is not effective, most probably because of a less effective recognition (≈20%) of nanoparticles by the macrophages. The reduced recognition is generally due to more diluted and scattered chemotactic signals as a consequence of (i) higher number concentration of nanoparticles (compared with micron-sized particles at the same dose) and (ii) fewer opsonin molecules available per particle. Conversely, since nanoparticles are more rapidly taken up by epithelial cells, they become less available to be phagocytized by macrophages. Macrophages are also located in the ciliated airway but their role in nanoparticle clearance is probably less essential compared with mucociliary clearance (Brain, 1992).

2.7.4.2.4 Translocation into Cells, Blood and Lymph

This process consists of transcytosis of the particles across the epithelia or into the epithelial cells of the respiratory tract into the interstitium and then to lymph and blood. As described before, translocation to the lymphatic system can be promoted by macrophage uptake. The delivery can be protein-mediated, requiring binding of certain proteins on the nanoparticle surface for identification by the receptors. The delivery can also be receptor-mediated transcytosis via caveolae, which have a diameter of 50–100 nm. Surface coating of the particles by lecithin and albumin can facilitate cellular uptake.
by pneumocytes and transcytosis across capillaries. Once internalized, nanoparticles can attach to DNA and even mitochondria in the nucleus.

When translocated to the systemic circulation, nanoparticles could lead to undesirable effects on the blood (e.g. accumulation in platelets) and other organs in the body. Some biological effects may consist of oxidative stress, inflammation, fibrosis, cytotoxicity, and immunologic responses.

Chronic Obstructive Pulmonary Disease – Current Concepts and Practice Surface area has been assumed as the single most essential particle dose parameter for the toxicity of nanoparticles. This is generally relevant for oxidative stress reactions and inflammatory, such as surface area of a catalyst (i.e. nanoparticles), that determines the oxidative reaction rate. therefore, oxidative stress consist of the formation of reactive oxygen species (ROS) from the particles including reactants such as polyaromatic hydrocarbons or transition metals. Nanoparticles containing drug, which do not include such reactants are however less likely to led to oxidative stress in the lungs. Biodegradable nanoparticles indeed exhibited significantly lesser inflammatory response in-vitro. Interestingly, translocation in the reverse direction with particles re-entrained from the interstitium or lung capillaries to the luminal side of the epithelium have been exhibited in animal models using rats and rabbits. Such back-translocation was suggested to be macrophage-mediated (Groneberg et al., 2003).

2.7.4.2.5 Neuronal Uptake

Translocation into afferent vagal nerves in the tracheobronchial airways has been suggested but still not well studied. Nanoparticles deposited in the nasal cavity have been found to be taken up by the olfactory lobe and translocated to the central nervous system. Therefore, such a neuronal uptake pathway is appropriate only if the drug nanoparticles are inhaled nasally. Existing data from toxicological and epidemiologic studies exhibited longer retention of inhaled nanoparticles in the lungs, but the use of these findings on nanoparticles is under investigation. In theory, inhaled nanodrug particles have the potential to be retained longer in the lungs followed by translocation and cellular uptake into the systemic circulation hence led to nanotoxicity. It can be estimated that the fate of the nanoparticles in the lungs, regarding the removal pathways, will based on the properties of both the drug molecule and the particle. Micron-sized aggregates of
nanoparticles will deposit by sedimentation in the tracheobronchial (TB) region where mucociliary clearance will operate to remove both the undissolved and dissolved drugs. Even discrete nanoparticles can deposit by diffusion in the tracheobronchial region (Bharti et al., 2013).

Drug nanoparticles deposited in the alveolar region will dissolve in the airway fluid and be absorbed. This is likely to be the case even for hydrophobic drugs with low aqueous solubility like inhaled corticosteroids due to the relatively low doses that are used. As a result of the low persistence of drug nanoparticles, mucociliary escalator and dissolution will likely be the main clearance pathways responsible for these particles before macrophage phagocytosis and other translocation pathways would initiate to play a significant role (Bharti et al., 2013).

2.8 PREPARATION OF POLYMERIC NANOPARTICLES FOR PULMONARY DRUG DELIVERY

The most commonly used techniques to obtain polymeric nanoparticles in pulmonary drug delivery are mentioned as follows

2.8.1 Spray Drying Technique

Spray drying is an recent pharmaceutical manufacturing process used to efficiently obtain respirable colloidal particles in the solid state. In this process, the feed solution is supplied at room temperature and pumped to the nozzle where it is atomized by the nozzle gas (Mosén et al., 2004; Stahl et al., 2002). The atomized solution is then dried by preheated drying gas in a special area to eliminate water moisture from the system, hence obtaining dry particles. This method is highly promising in manufacturing the particles of above 2-μm size. This method is investigated to have better control on particle formation and therefore can be easily translated to large scale production (Vidgren et al., 1987). This process is also appropriate for thermolabile materials, such as peptides and proteins, because mechanical high- energy input is prevented in this process. More essentially, spray-drying can led to uniform particle morphology (Patil and Sarasija, 2012; Jawahar and Reddy, 2012).
2.8.2 Spray Freeze Drying Method

It is an recent particle engineering method, which combines freeze-drying and spray-drying processing steps. It employs spraying the drug solution into liquid nitrogen as a freezing medium followed by lyophilization (Maa et al., 1999). This method obtain porous and light particles and high fine particle fraction with improved aerosol performance and approximate 100% yield at subambient temperatures. Thermolabile peptide and protein substances, such as plasmid DNA and insulin, can also be manufactured into dry powder inhalation products. therefore, this is an costly process restricted for only costly drug (Yu et al., 2004; Kuo and Hwang, 2004).

2.8.3 Supercritical Fluid Technology (SCF)

The general feature of the supercritical fluid process is the controlled crystallization of drugs from dispersion in supercritical fluids, carbon dioxide. This method has illustrated a wide range of application in manufacturing pulmonary inhalable formulations. Supercritical fluid technology can be divided into numerous classes (Tom and Debenedetti, 1991; Rehman et al., 2004). The most essential two are supercritical fluid extraction of emulsions (SFEE) and supercritical antisolvent precipitation (SAS). The principle mechanism of SAS is dependent on rapid precipitation when a drug solution is brought into contact with a supercritical CO2. SFEE is dependent on extraction of the organic phase in multiple emulsions or oil-in-water using supercritical CO2. Because lots of the drugs (eg, asthma drugs) are not soluble in CO2, SAS processes provide an excellent and easy way to obtain dry powder inhalation formulations. SFEE can give uniform crystalline drug nanoparticles, composite nanoparticles consisting nanosuspensions and polymeric materials and the drugs (Mansour et al., 2009; Steckel and Muller,1998).

2.8.4 Double Emulsion/Solvent Evaporation Technique

Respiratory nanoparticle production from solvent evaporation system / double emulsion involves preparation of oil/water (o/w) emulsions with subsequent elimination of the oil phase (ie, typically a volatile organic solvent) through evaporation. The emulsions are generally produced by emulsifying the organic phase consisting of the polymer, drug 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, producing drug-loaded polymeric nanoparticles (El-Baseir et al., 1997). By this technique, biodegradable polymers, consisting poly(glycolic) acid (PGA), poly(l-lactic acid) (PLA) and poly(lactide-co-glycolide) acid (PLGA), have been extensively determined as carriers for solid drug nanoparticles (Mansour et al., 2009; Jawahar and Reddy, 2012).

2.8.5 **Antisolvent Precipitation**

Crystalline drug particles with narrow size distribution could be manufactured by direct controlled crystallization. This process consists antisolvent precipitation of drug solution in a water-miscible organic solvent, followed by addition of a bridging solvent, which is partially miscible or immiscible with water. Growth-retarding stabilizing additives, such as hydroxylpropylmethylcellulose (HPMC), is generally added in the medium to yield particles with small size. The precipitated drug crystals shown low amorphous content and a high FPF (Rasenack et al., 2003; Chow et al., 2007).

2.8.6 **Coacervation or Ionic Gelation Method**

More research has been directed on the manufacture of nanoparticles using biodegradable hydrophilic polymers such as gelatin, chitosan and sodium alginate. Calvo and co-workers designed a method for manufacturing hydrophilic chitosan nanoparticles by ionic gelation. The method consist of a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to produce coacervates with a size in the range of nanometer. Coacervates are produced as a consequences of electrostatic interaction between two aqueous phases, whereas, ionic gelation contains the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature (Koradiya et al., 2012).

2.8.7 **Particle Replication In Nonwetting Templates**

PRINT is a top-down particle formation method developed by Dr. Joseph DeSimone and his group. This method is able to develop uniform populations of organic micro- and nanoparticles with complete control of shape, size and surface functionality, and allows the loading of delicate cargos such as peptides, proteins, small organic
therapeutics, contrast agents, oligonucleotides, siRNA, fluorophores and radiotracers. All these characteristics are important for controlling \textit{in vivo} biodistribution, transport, and drug-release mechanisms of nanoparticles (Gratton et al., 2007).

The principle of PRINT is to use a low surface energy fluoropolymeric mold that allow high-resolution imprint lithography, developing technique from the microelectronics industry, to produce a variety of organic particles. PRINT is therefore an adaptation of lithographic techniques found in the microelectronics industry to produce carriers of efficient size for use in nanomedicine (Gratton et al., 2008). Through the application of an appropriately designed master template, PRINT can efficiently alter particle size ranging from 20 nm to more than 100 μm. The particles shape may be cylinder, sphere, discs, and toroid with defined aspect ratios. PRINT is a promising and novel technology in nanoparticulate development and manufacture for use in pulmonary delivery (Gratton et al., 2008; Patil and Sarasija, 2012).

\textbf{2.8.8 Polymerization Method :} In this technique, monomers are polymerized to produce nanoparticles in an aqueous solution. Drug is incorporated either by adsorption onto the nanoparticles or by being dissolved in the polymerization medium after polymerization completed. The nanoparticle suspension is then purified to eliminate different surfactants and stabilizers employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This method has be investigated for forming poly alkylcyanoacrylate polybutylcyanoacrylate nanoparticles. Nanocapsule production and their particle size based on the concentration of the stabilizers and surfactants used (Koradiya et al., 2012).

\textbf{2.9 CHARACTERIZATION OF POLYMERIC NANOPARTICLES FOR PULMONARY DRUG DELIVERY}

\textbf{2.9.1 \textit{In vitro} Evaluation Methods for Pulmonary Drug Delivery Systems}

\textbf{2.9.1.1 \textit{In vitro} Characterization of Nanoparticulate Aerosol Systems}

Nanoparticles for pulmonary drug delivery may be characterized by comprehensive characterization methods (Hickey et al., 2007).

\textbf{2.9.1.1.1 Inertial Impaction :} It is the standard technique to determine the droplet or particle aerodynamic size from pharmaceutical aerosol delivery systems. It explain the
mechanism of the deposition of aerosol particles on the walls of an airway conduct. The impaction (obstruction) tends to take place where the airway direction alters. The big particles have high momentum (inertia) and are more likely to move in the initial direction of airflow, whereas those with low momentum adjust to the new direction of flow and pass around the obstruction (Williams et al., 1999). Inertial impaction provides Stokes’ law to investigate the aerodynamic diameter of particles being characterized. This has the benefit of incorporating density and shape effects into a single term. Cascade impactors applicable in sampling and collecting size-fractionated airborne particle samples and pharmaceutical aerosol particle samples for chemical or gravimetric analysis (Hickey et al., 2007; Barry and O’Callaghan, 1999).

2.9.1.1.2 X-Ray Powder Diffraction (XRPD): It is one of the most essential characterization tools applicable in materials science and solid state chemistry, since it directly determine the degree of molecular short-range vs long-range order (Newman and Byrn, 2003). Molecular long-range order is direct measure of crystallinity, while short-range order is direct measure of noncrystallinity such as amorphicity or liquid crystallinity. It provides information about the nature and extent of crystallinity and molecular order for solid-state materials, ie, how the atoms pack together in the crystalline state (Bates et al., 2006; Larhrib et al., 1999; Traini et al., 2008).

2.9.1.1.3 Differential Scanning Calorimetry (DSC): It is a potential and routinely applicable pharmaceutical thermal analytical technique for phase behavior study on hydrates, polymorphs, binding interactions, thermotropic, amorphocity and lyotropic phase transitions of pharmaceutical materials, including nanoparticles. Differential scanning calorimetry directly measures the loss and gain of enthalpy, that is, order-to-disorder (eg, melting) and disorder-to-order (eg, crystallization) phase transitions (Saleki-Gerhardt et al., 1994).

2.9.1.1.4 Scanning Electron Microscopy (SEM): It is used to locate the surface morphology of particles with a high magnification. The resolution permits recognition of specific surface characteristics and asperities that lead to mechanical interlocking (ie, structural cohesion) and that consist of high-energy “active” sites on the surface which promotes surface energetic properties and interparticulate interactions and ultimately promotes aerosol dispersion performance. Surface and interfacial/interparticulate forces
are of high importance in the properties of nanoparticles and in the properties of aerosols (Bunker et al., 2005).

Scanning electron microscopy is identified as unique tool in the visual examination of particles and their surfaces. The resolution is of the order of nanometers (magnifications in the range 20–100,000). A fine beam of electrons of medium energy (5–50 keV) scans a gold-palladium coated sample obtaining secondary electrons, light or cathodoluminescence, backscattered electrons and X rays. The latter permits for X-ray microanalysis for specific elements. Scanning electron microscopy is routinely applicable for imaging particles in the smaller size range and micron and for testing the surfaces of larger particles. The resolution permits recognition of specific surface geometric characteristics that are indicative of structural phenomena (Hickey et al., 2007).

2.9.1.1.5 Atomic Force Microscopy (AFM) : It is applicable in measurement of surface energy, surface nanotopographical imaging, and measurement of interparticulate forces. This type of microscopy works in mesoscopic scale resolution (10^{-6}–10^{-9} m) and quantify the individual particle and excipient interaction by direct force measurement in a different environmental conditions (Hickey et al., 2007; Sethuraman and Hickey, 2002).

2.9.1.1.6 Inverse Gas Chromatography (IGC) : It determines the surface free energy of bulk powders such as fibers, polymers, and composite materials. Inverse gas chromatography is a method for studying solids using gas chromatography principles. A solid analyte is coated onto or packed into a chromatography column and a series of polar and nonpolar probe gases are eluted. Interactions between the stationary phase and the gaseous probe molecules led to characteristic net retention volume, which is applicable in the estimation of the free energy of adsorption and other thermodynamic surface parameters (Hickey et al., 2007).

2.9.1.1.7 Karl Fischer Titration : It is applicable to analytically quantify small amounts of water present in the inhalation powder which has essential consequences on capillary condensation (ie, capillary force is an important interparticulate force in inhalation aerosol particles), solid-state properties, solid-state phase behavior, and solid-state stability of pharmaceutical particles in the solid-state (Mansour and Zografi, 2007).

2.9.1.2 In vitro Pulmonary Cell Culture Models
The lung may be anatomically divided into various parts: the trachea, the main bronchi, the terminal bronchioles, the conducting bronchioles and the alveoli (Agu et al., 2001; Groneberg et al., 2003). Drug delivery via inhalation can be absorbed throughout the conducting airway from the trachea down to the terminal bronchioles and ultimately the distal alveoli (Mathias et al., 1996; Forbes and Ehrhardt, 2005). The alveolar epithelium and airway of the lung, which have various cell types, provide barrier capability to drug absorption. In order to best understand the drug absorption phenomenon in various regions (eg, bronchial, tracheal and alveolar) of the respirable barriers, in vitro pulmonary cell culture models have been designed (Sakagami, 2006; Mobley and Hochhaus, 2001). The cell lines used include A549, A427, HBE14o, and the Calu line (-1, -2, and -3). These are immortal (continuous) cell lines and thus have different membrane structural characteristics compared with mortal cell lines which promotes drug absorption and efflux (Steimer et al., 2005).

2.9.2 In vivo Evaluation Methods for Pulmonary Drug Delivery Systems

2.9.2.1 Pharmacokinetic Study: Pharmacokinetic study after nebulization of colloidal dispersion of drugs Colloidal drug dispersions have been administered to male ICR mice via nebulization using a restraint-free small animal inhalation dosing chamber. Then, the mice were sacrificed at pre-determined time points post-dosing. Blood samples were taken by cardiac puncture, and the lungs were harvested for analysis by analytical instrument. From these experiments, serum (blood) and lung pharmacokinetics of colloidal drug dispersions may be estimated as reported previously (Vaughn et al., 2006; Yang et al., 2008; McConville et al., 2006).

2.9.2.2 Biodistribution Study: To assess the inhaled radiolabelled SLNs biodistribution, nanoparticles (200 nm) were radiolabeled with 99mTc and biodistribution studies were undertaken following aerosolisation and administration of a 99mTc-HMPAO-SLN suspension to a group of adult male Wistar rats. After delivery of radiolabeled SLNs, dynamic image acquisition was carried out in a gamma-camera, followed by static image collection. Then radiation counting was performed in organ samples, collected after the animals were sacrificed. From these experiments, biodistribution of SLNs may be traced and characterized (Videira et al., 2006; Videira et al., 2002).
2.9.2.3 Fluorescence/Bioluminescence Imaging Systems for Pulmonary Gene Delivery: Visualization and characterization of both the gene expression and the pulmonary delivery properties after dry powder inhalation in mice were recently reported by Mizuno and colleagues. It was exhibited that the pulmonary delivery and the gene expression may be characterized using fluorescence of indocyanine green (ICG) as a fluorescent label and the detection of luciferase activity, respectively, by using a nondestructive real-time \textit{in vivo} imaging system. They summarized that the dry powder consisting of both pCMV-Luc and ICG was applicable as a dual imaging system to gene expression in mice and visualize pulmonary delivery (Mizuno et al., 2009).

2.10 COMPONENTS OF PHARMACEUTICAL AEROSOLS

2.10.1 Propellant: It is responsible for producing the power pressure within the container and also expel the product when the valve is opened and foam production of the product or in the atomization (Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012).

2.10.1.1 For Oral and Inhalation

- Fluorinated hydrocarbons
- Di-chloro di-fluro methane (propellant 12)
- Di-chloro tetra-fluro ethane (propellant 114)

2.10.1.2 Topical Preparation

- Propane
- Butane
- Isobutane

2.10.1.3 Compound Gases

- Nitrogen
- Carbon di-oxide
- Nitrous oxide

2.10.2 Containers:

They should be stand at pressure as high as 140 to 180 psig (pounds per sq. inch gauge) at 1300 F. The containers are basically made up of metal or glass. But brittleness restricts the need of glass. If the pressure is less than 25psig and propellant content is less than 15% then glass may be used. Glass should be coated with plastic coating in two
layers if pressure is less than or equal to 33psig. Vinyl and Epoxy resins may be used as linings. Vinyl resins may produce strong lining but it will get destroyed by steam. But epoxy resins may be used as they are resistant to steam. A vinyl coating on which the epoxy coating is most appropriate for products having less PH. Choice of the material is based on- Pressure of the system, PH of the product, whether product is aqueous or not, physicochemical properties of preparation (Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012).

2.10.2.1 Metals

2.10.2.1.1 Tinplated Steel :

It is applicable for most aerosol as it is inexpensive, light and durable. It is steel that has been plated on both side with tin. Tin plated steel containers are of two types-

(a) Two pieces container body, consisting of a drawn cylinder, the base of the container, is held in place with double seam.

(b) The three piece container has aside seam the base being attached as for two piece container, the top has a 1 inch opening and is joined to body by double seaming.

2.10.2.1.2 Aluminium : Aluminium containers are highly resistant to corrosion than tinplated steel. Aluminium container are developed by an extrusion process and therefore have no seam. Aluminium is subjected to corrosion by alcohol and water.

2.10.2.1.3 Stainless Steel :

It is resistant to corrosion and no coating is required. It may withstand high pressure. Therefore they are expensive.

2.10.2.2 Glass

Glass containers are frequently coated with plastics. The coating provides protection from impact. Glass has advantage of being transparent so contents can be viewed. Glass is virtually inert.

2.10.2.3 Plastic

Not widely applicable for aerosol container. Polyethylene tetra phthalate (PET) container as needed for some non pharmaceutical products.
2.10.3 Valves:

Valves delivers the drug in desired form. They also give proper amount of medication. There are two types of valves are present which are metering valve and continuous spray valve. Dispersing of potent medication at proper dispersion/spray approximately 50 to 150 mg ±10% of liquid materials at one time use of same valve (Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012) (Figure-1.11).

![Diagram of Valve Components](image)

**Figure 2.7: Valve components**

2.10.4 Actuator:

The actuator or adaptor which is fitted to the aerosol valve stem is a device which on depression or any other required movement opens the valve and directs the spray to the desired area. The design of the actuator which incorporates an orifice of varying size and shape and expansion chamber is very important in promoting the physical features of the foam or spray, particularly in the case of inhalation aerosols, where the active ingredient(s) should be delivered in the proper particle size range. A proportion of the active ingredient(s) is generally deposited on the inner surface of the actuator; the amount present is however less than the amount released by actuation of the valve. Different types of actuators like Foam actuators, Spray actuators, Solid steam actuators, Special actuators are used (Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012).
2.11 FORMULATION OF PHARMACEUTICAL AEROSOLS (Patel et al., 2012; Pokar et al., 2012):

Pharmaceutical aerosols consist of two important components

2.11.1 Product concentrate:

Product concentrate consist of ingredients or mixture of active ingredients and other such as antioxidants, solvents and surfactants.

2.11.2 Propellant:

It may be single or blend of different propellants are used. Blends of propellant used in a pharmaceutical formulation to obtain desired solubility features or different surfactants are mixed to give the proper HLB value for emulsion system. Propellant provides the desired solubility, vapor pressure & particle size. Parameters like chemical, physical, and pharmaceutical properties of active ingredients and site of application are considered.

2.11.3 Types of System:

2.11.3.1 Solution System:

It contains the vapour and the liquid. It is also known two-phase system. No other solvent is required, if the drug is soluble in the propellant. Based on the type of spray required, either propellant 12 is added or other propellants are also added. When less volatile substances are added to the system, three phase system is formed depending on the amount of water present. Dried particle is obtained on the use of A-70 and wetten particles on use of A-3 and A-17 (Pokar et al., 2012).

2.11.3.2 Water Based System:

If only water or large amount of water is present to solubilize the contents then it is known water based system. If spray form is needed, then active ingredient is dispersed and other solvents must be present in the form of emulsion and propellant will be external phase. As the water is not miscible in propellant, then generally three phase system results. To increase the solubility of propellants in water, ethyl alcohol may be added to the system. To form a uniform dispersion, surfactants may be used and those surfactants which are soluble to large extent in non polar solvents and soluble to smaller extent in water are mostly preferred. Ester produced between glycerol, glycol, and polyhydroxylic acid like palmitic, oleic, stearic acids may be used as surfactants.
The surfactants composition ranges between 0.5 to 2.0 and propellant composition ranges from 25 to 60%. The recent advancement is the aquasol valve. In aquasol dispenser system, the drug is dissolved in the water or the mixture of water and alcohol. Then the propellant layer is located on the top water layer. The solubility of the propellant enhances as the amount of alcohol increases and will become completely soluble if only alcohol is present. In aquasol, the vaporized propellant and product via different ducts reaches the actuator. Here the randomization takes place which provide an uniform spray (Pokar et al., 2012).

2.11.3.3 Suspension or Dispersion Systems:

There encounter a number of problems by the use of co-solvent. To overcome this, suspension system is produced. In this system, in the propellant or the blend of propellants, the drug particles are suspended. Surfactants are added to decrease the settling rate (Pokar et al., 2012).

2.12 TYPES OF INHALERS

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

2.12.1 Nebulizers
2.12.2 Meter dose inhalers
2.12.3 Dry powder inhalers

2.12.1 Nebulizers:

Nebulizers have been applicable for many years to cure asthma and other respiratory diseases. There are two general types of nebulizer, ultrasonic and jet nebulizers. The jet nebulizer works by the Bernoulli principle by which compressed gas (air or oxygen) passes through a narrow orifice creating an area of low pressure at the outlet of the adjacent liquid feed tube. This led to drug solution being drawn up from the fluid reservoir and shattered into droplets in the gas stream (Hess et al.,1989; Sterk et al., 1984). The ultrasonic nebulizer uses a piezoelectric crystal vibrating at a high frequency (generally 1–3 MHz) to generate a fountain of liquid in the nebulizer chamber; the higher the frequency, the smaller the droplets produced. On average, only 10% of the dose placed in the nebulizer is generally deposited in the lungs (McElvaney et al., 1991; David et al., 1998) (Figure-2.8).
The physical properties of drug formulations can have an influence on particle size and nebulization rates. The ionic strength, viscosity, pH, osmolarity and surface tension can avoid the nebulization of some formulations. If the pH is too less, or if the solution is hypo- or hyper-osmolar, the aerosol can induce coughing, bronchoconstriction and irritation of the lung mucosa. As well, high drug concentrations can reduce the drug output with some nebulizers (Khilnani and Banga, 2008; Amirav, 2004; Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012).

The advantages of nebulizers are as follows (Labiris and Dolovich, 2003)
- These have no specific inhalation method or need time consuming co-ordination.
- These aerosolizes many drug solutions.
- These delivers large doses.
- These are appropriate for people too sick and infants.

The disadvantages of nebulizer are as follows (Labiris and Dolovich, 2003)
- These are time consuming.
- These are bulky.
- These are Non-portable.
- Their Contents are easily contaminated.
- These are relatively costly.
- These show poor delivery efficiency.
- These led to drug wastage.
- These have large performance variation between operating conditions and different models.

### 2.12.2 Metered-dose inhalers:

The Metered-dose inhalers (MDI) are mainly applicable for aerosol delivery devices today. The Metered-dose inhalers emit a drug aerosol regulated by propellants, such as hydrofluoroalkanes (HFAs) and chlorofluorocarbons (CFC) through a nozzle at high velocity ($> 30$ m s$^{-1}$). Metered-dose inhalers deliver only a small fraction of the drug dose to the lung. Generally, only 10–20% of the emitted dose is deposited in the lung. The large particle size of the spray and high velocity causes approximately 50–80% of the drug aerosol to impact in the oropharyngeal region. Hand-mouth dis-coordination is another barrier in the optimal use of the MDI (Koradiya et al., 2012) (Figure-2.9).

![Metered-dose inhalers](image.png)

**Figure 2.9 : Metered-dose inhalers**

The delivery efficiency of Metered-dose inhalers based on inspiratory flow rate (IFR), patient's breathing pattern and hand-mouth coordination. Enhancement in IFR results in reduction of total lung dose deposition and penetration into the peripheral airways. Fast inhalations ($> 60$ l min$^{-1}$) led to a reduced peripheral deposition because the aerosol is rapidly deposited by inertial impaction in the oropharyngeal regions and conducting airway. When aerosols are inhaled gradually, deposition by gravitational sedimentation in peripheral regions of the lung is increased. Peripheral deposition has also been exhibited to enhance with a decrease in respiratory frequency and an increase in
tidal volume. As the inhaled volume is enhanced, aerosols are able to penetrate more peripherally into the lungs (Ruggins et al., 1993; Smith et al., 1998; Bronsky et al., 1987).

A period of breath holding on completion of inhalation allows particles which penetrate the periphery to be deposited in that region, instead of being exhaled during the expiratory phase. Hence, the optimal conditions for inhaling metered dose inhalers aerosols are from a beginning volume equivalent to the functional residual capacity, actuation of the device at the beginning of inhalation, IFR of <60 l min−1 followed by a 10-s breath-hold at the end of inspiration. (Khilnani and Banga, 2008; Amirav, 2004; Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012)

The advantages of Metered - dose inhalers are as follows (Labiris and Dolovich, 2003)

- These are Compact.
- These are Portable.
- These are Multidose (approximately 200 doses).
- These are less costly.
- These have Sealed environment (no degradation of drug).
- These provide Reproducible dosing.

The disadvantages of Metered - dose inhalers are as follows (Labiris and Dolovich, 2003)

- These require patient co-ordination and Inhalation method.
- These have large oral deposition.
- These consist of maximum dose of 5 mg.
- These are available for limited range of drugs.

2.12.3 Dry Powder Inhalers

Dry powder systems use a single drug or its blends with a appropriate carrier, most often as lactose for delivery to the lungs. The three predominant factors in this system include Carrier, Drug, and device. Delivery of medication with a dry powder inhalers (DPIs) need collaboration of breathing and minimum patient coordination following the actuation of the device. Dry powder inhalers (Figure-2.10) are small, portable devices that may be easily taken in a pouch or purse. Application of spacers is not needed in this system. In addition, Dry powder inhalers are devoid of environmentally injurious like CFC propellants, which are essential in MDI formulation (Ogden et al., 1996; Chawla et al., 1994; Telko and Hickey, 2005).
In the view of the necessary ban of CFCs application in MDIs by the United Nations, Dry powder inhalers have become significantly enhanced as a pulmonary drug delivery system over the precedent decade. The aerosol drug delivery has led to dramatic alterations in both inhaler device and formulation aspects. The inhaler devices are mainly attractive as dry powders. Dry powder exhibited the larger chemical stability than the liquids which are applicable in atomizers. On the other hand, formulation and production of dry powders for inhalation is not easy and challenging due to the potential physical instability of the powder (Koradiya et al., 2012, Khilnani and Banga, 2008; Amirav, 2004; Patel et al., 2012; Pokar et al., 2012; Lahkar, 2012).

![Dry powder inhaler](image)

**Figure 2.10 : Dry powder inhaler**

There are two types of devices

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

ii. **Multi-dose Devices**: Multi-dose device utilizes a circular disk that consist of either four or eight powder doses on a single disk. The doses are stored in separate aluminum blister reservoirs till just before inspiration.

The advantages of dry powder inhalers are as follows (Labiris and Dolovich, 2003)

- These are portable.
- These are compact.
• These are easy to use.
• These are breath actuated.
• These do not require hand-mouth co-ordination.

The disadvantages of dry powder inhalers are as follows (Labiris and Dolovich, 2003)
• Their respirable dose based on inspiratory flow rate.
• Humidity may cause powders to aggregate and capsules to soften.
• Dose lost if patient inadvertently exhales into the DPI.
• Most DPIs consist of lactose.

2.13 MANUFACTURING OF PHARMACEUTICAL AEROSOLS (Pokar et al., 2012; Lahkar, 2012)

Valve placer, Concentrate filler, Pressure filler, Purger and crimper, Leak test tank equipments are utilized for large scale of production.

Manufacturing procedure:
It includes two steps,
1. Concentrate preparation
2. Propellant is mixed

With assistance of the procedures that are previously accepted, the product is fabricated and then tested.

The filling of aerosols in to the containers can be done by two methods namely
1. Cold filling method
2. Pressure filling method

2.13.1 Cold Filling Method:

Low temperature range -34º C to - 40º C needed. Product concentrate is chilled and added to open container followed by chilled propellant, or the concentrate and propellant may be chilled together and mixture added to container. A valve is then crimped. Container is allowed to pass through a heated test bath as a check for container strength and leakage. This technique is not appropriate for aqueous product or for preparations that are adversely affected by low temperatures.

2.13.2 Pressure Filling Method:

A. Method-1
• The product concentrate is added to container at room temperature.
• The valve crimped into place.
• The propellant is then added under pressure through the valve stem or through the actuator and around the sealing gaskets.

B. Method-2
• Under the cap method: product concentrate is added to the container and valve place in a position.
• A seal is produced around the shoulder of the container and utilizing a vacuum, the valve cup is raised slightly from the can and propellant is added.
• The valve is then crimped into the place.

This technique is mainly prominent than cold filling technique as much of the formulations cannot be cooled to very low temperatures.

2.14 EVALUATION PARAMETERS OF PHARMACEUTICAL AEROSOLS
(Pokar et al., 2012)

2.14.1 Flame Projection:
This test predicts the effect of an aerosol formulation on the extension of an open flame. Product is sprayed for 4 sec. into the flame. Based upon the nature of formulation, the flame is extended, and exact length was measured with ruler.

2.14.2 Flash Point:
Measured by using standard Tag Open Cap Apparatus. In which Aerosol product is chilled to temperature of - 25 0 F and transferred to the test apparatus. Temperature of test liquid enhanced gradually, and the temperature at which the vapors ignite is considered as a flash point. It is estimated for flammable component, which in case of topical hydrocarbons.

2.14.3 Vapor Pressure:
Measured by pressure gauge. Difference in pressure predicts the presence of air in headspace. A can punctuating device is present for accurately determining vapor pressure.
2.14.4 Density:

It is measured by pycnometer or hydrometer. This technique is utilized for non-aerosol, modification to accommodate liquefied gas preparation. In which a pressure tube is fitted with metal fingers and hoke valve, which permits for the introduction of liquids under pressure. The hydrometer is placed in to the glass pressure tube. Appropriate sample is introduced through the valve to cause the hydrometer to rise half way up the length of the tube. The density may be read directly.

2.14.5 Moisture Content:

Gas chromatography method or Karl Fischer method has also been utilized for Identification of propellants: I.R spectrophotometry methods or Gas chromatography are utilized for identification of propellants.

2.14.6 Aerosol Valve Discharge Rate:

It is measured by taking an aerosol known weight and discharging the contents for known time utilizing standard apparatus. By reweighing the container after time limit has expired, the alternation in weight per time dispensed is discharge rate. It is denoted as gram per seconds.

2.14.7 Dosage with Metered Valves:

Reproducibility of dosage every time the valve is dispersed. Amount of medication actually received by the patient. Reproducibility has been measured by assay method. Another method is that, include accurate weighing of filled container followed by dispersing of several doses, container may reweighed, and difference in weight divided by No. of dose, gives the average dosage.

2.14.8 Particle Size Determination:

Cascade impactor operates on the projected through a series of nozzle and glass slides at high velocity, the large particles generally become impacted first on the lower velocity stages, and the smaller particles pass on and are collected at high velocity stages. These practical ranging from 0.1 to 30 micron. Modification made to improve efficacy.

2.14.9 Therapeutic Activity:

- For Inhalation Aerosols: It is based on the particle size.
- For Topical Aerosols: It is applied to test areas & adsorption of therapeutic ingredient is measured.
2.14.10 Toxicity:
- For Inhalation Aerosols: exposing test animals to vapor sprayed from Aerosol container.
- For Topical Aerosols: Irritation & Chilling effects are measured.

2.15 SYSTEM SPECIFIC REVIEW

1) Pilcer et al., (2009) demonstrated that utilizing spray-drying and high-pressure homogenization methods, novel formulations were designed for manufacturing dry powder for inhalation, consists of a mixture of micro- and nanoparticles in order to increase lung deposition. Particle size analysis was carried out by laser diffraction. Spray-drying was applied in order to retrieve nanoparticles in dried-powder state from tobramycin nanosuspensions. The aerosolization properties of the various formulations were characterized by a multi-stage liquid impinger. Suspensions of nanoparticles of tobramycin consisting Na glycocholate at 2% (w/w) relative to tobramycin content and presenting a mean particle size about 200 nm were obtained. The results from the spray-dried powders exhibited that the presence of nanoparticles in the formulations improved particle dispersion characteristics during inhalation. The fine particle fraction (percentage of particles below 5 micron) enhanced from 36% for the raw micronized tobramycin material to about 61% for the major effective formulation. These new nanoparticles-consisting tobramycin DPI formulations, dependent on the application of very low level of excipient and presenting high lung deposition characteristics, offer very essential perspectives for improving the delivery of drugs to the pulmonary tract.

2) Chattopadhyay et al., (2007) utilized a continuous supercritical carbon dioxide extraction process to obtain solid lipid nanoparticles suspensions for pulmonary delivery. In this phenomenon, supercritical carbon dioxide was utilized to extract organic solvent from an oil in water emulsion consisting of one of three lipids (tristearin, tripalmitin, or gelucire 50/13), and one of two model drugs (ketoprofen or indomethacin). One of the aforementioned lipids and a selected drug was dissolved in chloroform with a soy lecithin surfactant, then dispersed into an aqueous solution consisting of sodium glycocholate and homogenized under high pressure to obtain the emulsion. This reportedly created an
emulsion with a mean droplet size ranging between 30 and 100 nm, which was incorporated into an extraction column counter currently to a stream of supercritical carbon dioxide. The scCO₂ extracted the organic solvent from the dispersed droplets, leaving behind solid lipid, drug-containing particles in an aqueous suspension. This processing technique obtained nanoparticles with a volume mean diameter between 10 and 30 nm, and a drug loading efficiency between 80% and 90% for the gelucire particles and 10% for the tripalmitin particles. Nebulized droplets were obtained from the suspensions within an aerodynamic diameter range between 2 and 4 μm, which is within the respirable range.

3) Xia et al., (2004) demonstrated that liposomal and polymeric nanoparticles loaded with doxorubicin and spray dried with lactose in the manner explained above were more effective at killing chemo-resistant and chemo-sensitive lung cancer cells than unloaded nanoparticles and pure drug.

4) Dailey et al., (2003) capitalizes on both solvent displacement and ionotropic complexation techniques developed a novel branched polyester, diethylaminopropyl amine-poly(vinyl alcohol)-grafted-poly(lactide-co-glyco-lyde) (DEAPA-PVAL-g-PLGA), which consist of a cationic tertiary amine group and a degradable PLGA backbone. The ratio of polymers had a great impact on both the zeta potential and the particle size. As the concentration of CMC enhanced, the zeta potential reduced, and as the zeta potential approached neutrality, the particle size enhanced. The authors suggest that this is most likely due to enhanced particle agglomeration as the surface charge approaches neutralit, thereby diminishing Coulombic forces between particles. Additionally, as the concentration of CMC enhanced further, the zeta potential continued to reduce, and the particle size began to reduce. The authors found that the enhanced hydrophilicity of nanoparticles enhanced the of degradation as compared to pure PLGA particles. They also observed that the more neutral the zeta potential of the particles, the less fast they degraded over the time, presumably due to enhancement in lipophilicity. This study also reported an enhanced stability in negative charge particles after nebulization.
5) Shi et al., (2007) had shown that oppositely charged nanoparticles may be assembled to form larger, low density microstructures appropriate for pulmonary delivery. In this study PLGA nanoparticles were obtained utilizing a solvent displacement procedure. Briefly, PLGA was dissolved in methanol and acetone, then injected at controlled rate into an aqueous solution containing either dissolved poly(N-vinyl formamide) (PVAm) or poly(ethylene-maleic anhydride) (PEMA) under homogenization. The residual acetone was then evaporated, and the remaining particles were collected. Particles obtained in the PEMA solution were around 300 nm in diameter with a zeta potential of around – 50 mV, and the particles obtained in PVAm solution were slightly larger at around 500 nm and with zeta potential of around +30 mV. The difference in zeta potential was due to the charges of the coating polymers, PVAm a polycation and PEMA being a polyanion. A suspension of PVAm-coated particles were then injected into a suspension of PEMA-coated particles under homogenization to destabilize the colloid and led to particle flocculation; induced by the ionic interactions between particles in suspension. The authors determined that lyophilized samples of the nanoparticles flocculates have aerodynamic diameters between 2 and 4 μm, making them ideally sized for pulmonary delivery. Additionally, SEM images exhibited that the nanoparticles flocculates had irregular geometries and were largely porous structures, which would enhance particle aerosolization in a DPI.

6) Pandey et al., (2003) demonstrated that poly(lactic-co-glycolic) acid (PLGA) nanoparticles were produced utilizing a multiple emulsion/solvent evaporation method similar to those previously. The authors obtained nanoparticles of isoniazid, rifampicin or pyrazinamide, which are utilized to cure tuberculosis. Briefly, aqueous drug solution was emulsified in a dichloromethane solution consisting of PLGA polymer via probe sonication. The emulsion was then added to an aqueous containing polyvinylalcohol and sonicated. The resulting solution was then stirred overnight to eliminate the organic phase, and particles were collected by centrifugation. This method obtained particles with sizes ranging between 180-300 nm and a drug encapsulation efficiency between 50% and 70%, based on the drug used. The particle suspensions were then nebulized, reportedly obtaining droplets between 1.1 and 2.1 μm, which is within the respirable range.
7) Broichsitter et al., (2009) analyzed that the application of colloidal carrier systems for pulmonary drug delivery is an upcoming field of interest in nanomedicine. The aim of this study was to relate the pulmonary absorption and distribution characteristics of the hydrophilic model drug 5(6)-carboxyfluorescein (CF) after aerosolization as solution or entrapped into nanoparticles in an isolated rabbit lung model (IPL). CF-nanoparticles were obtained from a new class of biocompatible, fast degrading, branched polyesters by a modified solvent displacement technique. Physicochemical properties, encapsulation efficiency, morphology, stability of nanoparticles, \textit{in vitro} drug release to nebulization, aerosol characteristics as well as pulmonary dye absorption and distribution profiles after nebulization in an IPL were estimated. In summary, the data suggest that inhalative delivery of biodegradable nanoparticles can be a viable approach for pulmonary drug delivery. However, a targeting effect to the lung tissue is claimed.

8) Bhavane et al., (2003) developed a novel type of drug delivery vehicle consisting of liposomal nanoparticles covalently linked by enzymatically labile spacers. In this study, liposomes were manufactured by extruding a suspension of dissolved, hydrated lipids. The lipid mixture involved cholesterol, DPPC, and 1,2-disteryl-sn-glycero-3-phosphorylEtanolamie-poly(ethylene glycol)-amine (DSPE-PEG-NH$_2$), which consist of an amine group appropriate for conjugation. This phenomenon reportedly prepared liposomes between 80 and 195 nm in size. Liposomes were loaded with ciprofloxacin with 90\% efficiency. Agglomeration was induced using dimethyl 3, 3’-dithiobispropionimidate 2HCl (DTBP), which is a homo bifunctional imidoester capable of reacting with primary amines and also consist of thiol-cleavable disulfide bonds, forming it enzymatically labile. The authors investigated that at low PH, smaller agglomerates were obtained due to the restricted activity of the linker. The agglomerates illustrated slower release kinetics than the unagglomerated liposomes and a burst effect was observed at time points when dithiothretion (a disulfide bond reducing agent) was added to the dissolution medium. Upon nebulization, the agglomerated particles were reported to retain the encapsulated drug and nebulized droplets had aerodynamic diameters between 1 and 5 \(\mu\)m, putting them within the respirable range. The study reports that controlled agglomeration of liposomal nanoparticles may be obtained and that these particles may be nebulized into respirable range.
9) Nyambura et al., (2009) studied nanoparticles delivered from pressurized metered dose inhalers (PMDIs) potentially offer a means of precisely delivering proteins to the lung. Nanoparticles containing the model protein lysozyme have been obtained using nanoprecipitation and microemulsion methods. Freeze-dried water in oil emulsions, with chloroform as the organic solvent, followed by washing of excess surfactant (lecithin) led to the fabrication of lactose nanoparticles having approximately 300 nm mean size. Substitution of lactose with lysozyme caused a significant enhancement in the mean size of nanoparticles (645-750 nm). This may have been due to the surface activity of lysozyme which changed the emulsification characteristics. The retained biological activity of lysozyme enhanced with increased lactose concentration in the formulation and approximately 99% biological activity was retained when 20% (w/w) lactose was utilized (Nyambura et al., 2009).

10) Sham et al., (2004) used spray dried lactose with either poly(butyl)cyanoacrylate or gelatin nanoparticles to obtain particles for pulmonary delivery. Particle sizes were found to be around 170 nm and 240 for the poly(butyl)cyanoacrylate and gelatin nanoparticles respectively. Particles were then spray dried using a parallel flow nozzle, which sprays solution and drying air in the same direction to atomize the drug solution. The authors indicated producing powders with sizes of 2.50, 2.59 and 2.60 μm for pure lactose, lactose and gelatin nanoparticles, and lactose and poly(butyl)cyanoacrylate nanoparticles, respectively. The inclusion of the nanoparticles in the formulations did not necessarily affect the particle sizes, as measured by a student’s t-test. This study illustrated that polymeric nanoparticles may be incorporated into larger particles with appropriate sizes for pulmonary delivery. The authors have also estimated that these particles have applications as ‘cluster bombs’ that may be used to deliver chemotherapeutics to lung cancer cells.

11) Yamamoto et al., (2007) combined emulsion/ solvent evaporation nanoparticles preparation with a modified form of spray drying to obtain microparticle appropriate for inhalation. Briefly, the drug 6-coumarin and PLGA were dissolved in an ethanol and acetone mixture and injected into an aqueous polyvinylalcohol(PVA) solution while being stirred at 400 rpm, obtaining particles approximately 250 nm in diameter after
evaporation of the organic phase. Lyophilized powder was then suspended in water consisting of dissolved mannitol and spray dried in a fluidized bed granulation system. In this phenomenon, solution is sprayed from the bottom of the reactor into the granulation chamber and the resulting mist was dried by heated air. Dried particles were then entrapped onto a backdrop filter and redispersed into the granulation chamber with a pulsed air jet. The authors assumed that the particles were granulated by the coalescence of dry and wet particle collision within the granulation chamber.

12) **Dailey et al., (2003)** investigated the effect of nebulization technology and nanoparticles characteristics on the features of aerosol generation. Suspensions of biodegradable nanoparticles containing commercially available poly(lactide-co-glycolide) and novel comb polymers were nebulized with a ultrasonic, jet, and piezo-electric crystal nebulizer. The effects of the nanoparticles suspensions on the nanoparticles size as well as the aerosol droplet size before and after nebulization, were evaluated via laser diffraction. While the individual nanoparticles suspension exhibited no clinically relevant influence on aerosol droplet size, as compared to control experiments, an increased nanoparticles aggregation within the droplets was observed. This aggregation was further evaluated by scanning electron microscopy and fluorescence. Dependency of aggregation on nanoparticles characteristics and nebulizer technology was noted. Nanoparticles showing the highest surface hydrophobicity were particularly susceptible to aggregation when nebulized with a jet nebulizer. Aggregation was decreased with nanoparticles showing a more hydrophilic surface or when utilizing ultrasonic nebulizer. The authors summarized that the biodegradable nanoparticles contained in the suspensions did not affect the aerosol droplet size in a clinically relevant manner, therefore, both the technique of aerosol generation and the nanoparticles characteristics influence nanoparticles aggregation occurring during aerosolization.

13) **Bivas-benita et al., (2004)** dissolved polyethylenimine (PEI) and poly(lactic-co-glycolic acid (PLGA) in dichloromethane, acetone and Tween-80. This solution was injected into an aqueous phase consisting of poloxomer-188 as a surfactant, and stirred gradually to evaporate the organic phase. The resulting particles were filtered and complexed with DNA. To achieve this, a nanoparticles suspension was added to a DNA
solution and vortexed. Particles were created at varying PLGA-PEI ratios and PEI-DNA ratios. This technique consistently obtained particles with sizes between 207 and 231 nm, regardless of PEI-DNA and PLGA-PEI ratios used. The authors also predicted that the zeta potential was not affected by the PLGA-PEI ratios, but was based on the PEI-DNA ratio.

14) Grenha et al., (2008) obtained nanoparticles utilizing an ionotropic gelation technique. Tripolyphosphate (TPP) and Chitosan were dissolved in aqueous solutions and mixed under mild stirring to spontaneously precipitate nanoparticles. Insulin was dissolved in 0.01 M NaOH solution and added to the Tripolyphosphate solution before being added to the chitosan solution. Particles with sizes ranging from 300 to 500 nm and with zeta potentials between +32 and +45 mV were obtained in this manner. The authors reported that enhancing the chitosan to TPP mass ratio reduced the process yield (mass of particles produced over total mass), but enhanced the zeta potential and size of the particles. The glycosidic linkages in chitosan), suggesting that they will degrade in the pulmonary epithelium and release their drug contents. These particles were then incorporated into microparticles by incubating them in mannitol and lactose excipient solutions and spray drying. This phenomenon obtained microparticles with aerodynamic diameters between 2 and 3 μm, which are appropriate for pulmonary delivery. The microparticles fastly dissolved in aqueous solution, leaving behind a suspension of nanoparticles. The authors summarized that these particles could potentially be utilized to effectively deliver therapeutic macromolecules to the lungs and promote pulmonary absorption.

15) Ely et al., (2007) fabricated poly(butyl)cyanoacrylate nanoparticles as a model particle. The resulting nanoparticles were then suspended in a aqueous solution consisting of citric acid, polyethylene glycol 6000, sodium carbonate, and L-leucine. The suspension was then spray dried to obtain particles with an aerodynamic diameter of 2.17 μm . The authors predicted that, when dissolved in water, the effervescent particles illustrated more active release of nanoparticles as compared to lactose control particles, as measured by the large (~30 μm ) nanoparticles-filled bubbles that were reported with the effervescent powders. This type of formulation could reduce the residence time of the
microparticles in the lung tissue, potentially reducing the chance of being phagocytized by alveolar macrophages. Hence, possibly increasing the bioavailability of the drug.

2.16 POLYMER SPECIFIC REVIEW

2.16.1 Polymers Used To Prepare Polymeric Nanoparticles

2.16.1.1 Gelatin: Gelatin is a heterogeneous mixture of water-soluble proteins of high average molecular masses, present in collagen. The proteins are extracted by boiling skin, ligaments, tendons, bones, etc. in water. Type A gelatin is obtained from acid-cured tissue and Type B gelatin is obtained from lime-cured tissue. The chemical structure of gelatin is shown in (Figure-2.11).

2.16.1.1.1 Physical Properties:

2.16.1.1.1 Isoelectric point (pI): The charge on gelatin molecule and its isoelectric point are primarily due to the amino, carboxyl, and guanidino groups on the side chains. Type A gelatin has 78-80 millimoles of free carboxyl groups per 100 g of protein and pI of 7.0 - 9.0; type B has 100-115 millimoles of free carboxyl groups per 100 g of protein and a pI of 4.7-5.2. The pH of a 1.5% solution at 25 ° C is 3.8-5.5 for Type A and 5.0-7.5 for Type B.

2.16.1.1.2 Solubility: Gelatin is soluble in acetic acid and glycerol, and more soluble in hot than in cold water. It is practically insoluble in numerous organic solvents such as alcohol, carbon disulfide, chloroform, carbon tetrachloride, benzene, ether, acetone, and oils.

![Chemical structure of gelatin](image)

Figure 2.11: Chemical structure of gelatin
2.16.1.2 Storage/Stability: Dry gelatin stored in airtight containers at room temperature remains unaltered for many years. When heated at 100 °C in the presence of air it swells, becomes soft, and disintegrates to a carbonaceous mass with evolution of ammonia and pyridine bases. Below 35-40 °C gelatin swells in and absorbs 5 -10 times its weight of water to produce a gel. Sterile solutions of gelatin, stored cold, remain unaltered indefinitely, but at elevated temperatures rupture or hydrolysis of peptide bonds takes place, enhancing the number of free amino groups. Viscosity and Gel strength gradually weaken in pH, bacterial action and proteolytic enzymes (Young et al., 2005; Martindale, 1989).

2.16.1.3 Applications: It is utilized in coating cell culture plates to improve cell attachment for a variety of cell types, addition to PCR to assist stabilize Taq DNA polymerase, and applicable as a blocking reagent in ELISA, Western blotting, and immunohistochemistry. In bacteriology, gelatin can be utilized as a component of culture media for species differentiation. Additionally, as a biocompatible polymer, gelatin has been utilized as a delivery vehicle for the release of bioactive molecules and in the generation of scaffolds for tissue engineering applications (Young et al., 2005; Martindale, 1989).

Industrial applications involves the use of gelatin as a thickener, stabilizer, and texturizer in foods and in the manufacture of adhesives, rubber substitutes, cements, lithographic and printing inks, artificial silk, plastic compounds, photographic plates and films, matches, and light filters for mercury lamps. In the pharmaceutical industry, gelatin is utilized as a encapsulating agent, suspending agent, and tablet binder; and in veterinary applications it is utilized as a hemostatic sponge and plasma expander.

2.16.1.2 Poly Lactic-co-Glycolic Acid (PLGA):

Poly(D,L-lactide-co-glycolide) (PLGA) is the frequently used biodegradable polymer for producing nano/microparticles encapsulating therapeutic drugs in controlled release (CR) applications. PLGA dependent drug delivery devices have numerous advantages over the conventional devices. One of the advantage is the extended release rates of drugs up to days, weeks or months. Other reasons for the widespread application of PLGA are its biocompatibility, its biodegradability, and the fact that PLGA has been
approved by FDA (Food and Drug Administration). Various active pharmaceutical ingredients such as analgesics, anti-cancer drugs, antibiotics and macromolecular drugs such as peptides, proteins, vaccines, genes, antigens, vascular endothelial growth factors, human growth factors, etc., are successfully incorporated into PLGA or PLGA based drug delivery devices. As a result, these systems in general may be utilized to provide targeted (tissue or cellular) delivery of drugs, which localized effect represents also an essential benefit. They improve bioavailability, sustain release of drugs or solubilize drugs for systemic delivery. Drug delivery using PLGA or PLGA based polymers is an attractive area with different opportunities for further research and developmental work (Makadia and Siegel, 2011). (Figure-2.12)

2.16.1.2.1 Physicochemical Properties: Poly(lactic acid) (PLA) is a linear aliphatic thermoplastic polyester, obtained by polymerization of lactide, a cyclic dimer derived from lactic acid. It is a chiral molecule and may be obtained as poly (D-lactide), poly (L-lactide), and the racemic poly (D,L-lactide). Poly(lactic acid) is soluble in common organic solvents. Poly(glycolic acid) (PGA) is the simplest linear, aliphatic polyester. Since Poly(glycolic acid) is highly crystalline, it has a high melting point and low solubility in organic solvents. PGA’s high crystallinity is due to its chemical structure lacking the methyl side groups of the Poly(lactic acid). PLGA is a copolymer of lactide and glycolide, (Figure-8) which is synthesized by means of random ring-opening and when PGA randomly copolymerized (30-50 %) with Poly(lactic acid), resulting copolymer (PLGA) retains physical properties more rapidly amenable to processing (those of low-melting thermoplastic with good solubility in common solvents). The

![Structure of Poly Lactic-Co-Glycolic Acid](image-url)
The degradation rate of PLGA is rapid than that of Poly(lactic acid) because of the component glycolic acid in the backbone, and in addition the degradation rate may be adjusted by changing the amounts of lactic acid and glycolic acid (Makadia and Siegel, 2011).

The Tg (glass transition temperature) of the PLGA copolymers are above the physiological temperature of 37ºC and thus, they are glassy in nature. They have a fairly rigid chain structure which provides them significant mechanical strength to be manufactured as drug delivery devices. Tg of PLGAs reduce with decrease of their lactide content in the copolymer composition and with reduction in their molecular weight (Oya and Emine, 2012).

PLGA polymers are exposed to physical stress when using as drug delivery devices so they must have a considerable mechanical strength. Various factors like copolymer composition (lactide/glycolide ratio), the molecular weight, crystallinity and geometric regularity of individual chains significantly affect the mechanical strength of the polymer (Park et al., 2005).

2.16.1.2.2 Biological Properties: PLGA is one of the main successfully used biodegradable polymer because it led to hydrolysis in the body to obtain the biocompatible and biodegradable metabolite monomers (glycolic acid and lactic acid) that are eventually eliminated from the body by the citric acid cycle (Figure – 2.13). Polymer biodegradation products are produced at a very gradual rate and thus, they do not affect the normal cell function. Since the body effectively deals with these two monomers, there is very minimal or no systemic toxicity associated by using PLGA for drug delivery or biomaterial applications (Anderson and Shive, 1997).

![Figure 2.13: Hydrolysis of Poly Lactic-Co-Glycolic Acid.](image-url)
2.16.1.3 Bovine Serum Albumin

Bovine serum albumin (also known as BSA or "Fraction V") is a serum albumin protein obtained from cows. It is generally utilized as a protein concentration standard in laboratory experiments. Bovine albumin is a single polypeptide chain containing approximately 583 to 595 amino acid residues and no carbohydrates. At pH 5-7 it consists of 17 intrachain disulfide bridges and 1 sulfhydryl group.1,2,4 Although the conformation of Bovine serum albumin is considered to be same to Human serum albumin(Figure-1.10) due to 76% of amino acid sequence homology, the three-dimensional (3-D) structure of Bovine serum albumin has yet to be evaluated. The biological function of a protein based on its conformation. The main informative technique to study the 3-D structure of proteins is X-ray crystallography. The X-ray crystallography, which provides information regarding the atomic distances of a crystallized compound, is not always useful because not all proteins may be rapidly crystallized (Huang and Kim, 2004).

![Figure 2.14: Structure of albumin](image)

The most essential physiological function of serum albumin is to manage the pH of blood and osmotic pressure and transport a large variety of exogenous and endogenous compounds involving metal, fatty acids, steroids, amino acids and drugs. Because of these extraordinary features, albumins from different sources have gained extensive industrial and biomedical applications as well as research interest (Huang and Kim, 2004).
2.16.1.3.1 Composition of Bovine Serum Albumin

The full-length Bovine serum albumin precursor protein is 607 amino acids in length. An N-terminal 18-residue signal peptide is cut off from the precursor protein upon secretion, thus, the initial protein product consists of 589 amino acid residues. An additional 4 amino acids are broken down to yield the mature Bovine serum albumin protein that consists of 583 amino acids.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Position</th>
<th>Length</th>
<th>MW Da</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full length precursor</td>
<td>1 – 607</td>
<td>607</td>
<td>69,324</td>
</tr>
<tr>
<td>Signal peptide</td>
<td>1 – 18</td>
<td>18</td>
<td>2,107</td>
</tr>
<tr>
<td>Propeptide</td>
<td>19 – 22</td>
<td>4</td>
<td>478</td>
</tr>
<tr>
<td>Mature protein</td>
<td>25 – 607</td>
<td>583</td>
<td>66,463</td>
</tr>
</tbody>
</table>

2.16.1.3.2 Physical Properties of Bovine Serum Albumin

- Molecular weight: 66,463 Da (= 66.5 kDa)
- Number of amino acid residues: 583
- Extinction coefficient of 43,824 M⁻¹cm⁻¹ at 279 nm
- Isoelectric point in water at 25 °C: 4.7
- Dimensions: 140 × 40 × 40 Å (prolate ellipsoid where a = b < c)
- Optical Rotation: \([\alpha]_{259}^\circ: -61^\circ; [\alpha]_{264}^\circ: -63^\circ\)
- pH of 1% Solution: 5.2-7
- Sedimentation constant, \(S_{20,W} \times 10^{13}: 4.5\) (monomer), 6.7 (dimer)
- Stokes Radius \((r_s): 3.48\) nm
- Partial specific volume, \(V_{20}: 0.733\)
- Diffusion constant, \(D_{20,W} \times 10^7\) cm²/s: 5.9
- Intrinsic viscosity, \(\eta: 0.0413\)
- Refractive index increment (578 nm) × 10⁻³: 1.90
- Frictional ratio, \(f/f_0: 1.30\)
- Optical absorbance, \(A_{279}^{\text{nm}}\) g/L: 0.667
• Mean residue ellipticity: 21.1 [θ]_{209\ nm}; 20.1 [θ]_{222\ nm}
• Mean residue rotation, [m']_{233}: 8443
• Estimated b-form, %: 18
• Estimated a-helix, %: 54

2.16.1.3.3 Applications of Bovine Serum Albumin

Bovine serum albumin has large number of biochemical applications consisting of immunoblots, ELISAs (Enzyme-Linked Immunosorbent Assay), and immunohistochemistry. It is also utilized as a microbial culture and nutrient in cell. In restriction digests, Bovine serum albumin is utilized to stabilize few enzymes during digestion of DNA and to avoid adhesion of the enzyme to pipet tips, reaction tubes, and other vessels. This protein does not harm other enzymes that do not require it for stabilization. Bovine serum albumin is also generally used to measure the quantity of other proteins, by comparing an unknown quantity of protein to known amounts of Bovine serum albumin. Bovine serum albumin is utilized because of its stability to enhance signal in assays, its lack of effect in many biochemical reactions, and its low expense, since wide quantities of it may be rapidly purified from bovine blood, a byproduct of the cattle industry (Huang and Kim, 2004).

2.17 DRUG SPECIFIC REVIEW

2.17.1 Terbutaline Sulfate :

2.17.1.1 Physical Properties : It is a white to gray-white crystalline powder. It is odorless or has a faint odor of acetic acid.

2.17.1.2 Solubility: It is soluble in in 0.1N hydrochloric acid and water, slightly soluble in methanol, and insoluble in chloroform.

2.17.1.3 Molecular weight: Its molecular weight is 548.65.

2.17.1.4 Chemical Formula: Terbutaline sulfate is (±)-α-[(tert- butylamino)methyl]-3,5-dihydroxybenzyl alcohol sulfate (2:1) (salt) (Kathleen, 1999).
2.17.1.5 Molecular Formula: \((C_{12}H_{19}NO_3)_2 \cdot H_2SO_4\)

2.17.1.6 Structural Formula:

![Chemical structure of Terbutaline sulfate](image)

Figure 2.15: Chemical structure of Terbutaline sulfate

2.17.1.7 Mechanism of action

Terbutaline led to bronchodilation by direct stimulation of \(\beta_2\) adrenergic receptors located in bronchial smooth muscles.

2.17.1.8 Pharmacokinetics: Terbutaline is differently absorbed from the gastrointestinal tract and about 60% of the absorbed dose undergoes first-pass metabolism by sulfate (and some glucuronide) conjugation in the gut wall and the liver. It is generally excreted in the urine partly as unchanged terbutaline and partly as the inactive conjugates, the ratio based upon the route by which it is administered. The half-life is found to be about 3 to 4 hours (Kathleen, 1999).

2.17.1.9 Adverse Effect:

1. Pulmonary oedema:
Pulmonary oedema has occurred in women given beta\(_2\) agonists, involving terbutaline, for premature labour.

2. Tolerance:
As with other beta\(_2\) agonists there is some evidence that tolerance may produce to terbutaline when it is utilized regularly.
(3) Tooth erosion.

The pH of few inhaled powder formulations of bronchodilator (including terbutaline) and anti-inflammatory drugs was reported to be below 5.5, and it was predicted that this might contribute to the dissolution of enamel surfaces of teeth.

(4) Other includes cardiac arrhythmias, Palpitation, angina pectoris, tachycardia can be precipitated, dizziness, Anxiety, headache, sweating, remor, nausea and vomiting.

2.17.1.10 Contraindications : Hypersensitivity to cardiovascular disease, sympathomimetics, hyperthyroidism.

2.17.1.11 Indications : To cure acute bronchospasm in children and adults, to arrest uncomplicated premature labour, urticaria, hypoglycaemia and Systemic capillary leak syndrome

Terbutaline Sulfate Inhalation aerosol : It is a suspension of microfine Terbutaline Sulfate in appropriate propellant in a pressurized container. It consist not less than 90.0 percent and not more than 110.0 percent of the labeled amount of \( \text{(C}_{12}\text{H}_{19}\text{NO}_{3})_2\text{H}_2\text{SO}_4 \).

2.17.1.12 UV Spectroscopic Method for Estimation of Terbutaline Sulfate (Oza et al., 2012)

2.17.1.12.1 Terbutaline sulphate (TBS) standard stock solution: (100 μg/ml)

A 10 mg of terbutaline sulfate standard was weighed and transferred to a 100 ml volumetric flask. 70 ml of water was transferred to this volumetric flask and sonicated for 10 min. The flask was shaken and volume was made up to the mark with water to give a solution consisting of 100 μg/ml terbutaline sulfate.

2.17.1.12.2 Selection of Analytical Wavelength: 20 - 60 μg/ml solutions of terbutaline sulfate were produced in water and spectrum was recorded between 200-400 nm. terbutaline sulfate exhibited \( \lambda_{\text{max}} \) at wavelength at 279 nm.

2.17.1.12.3 Calibration curve for the Terbutaline Sulfate: Suitable volume of aliquot from standard terbutaline sulfate stock solution was transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with the water to produce concentration of 20, 30, 40 50 and 60μg/ml. The curve of each solution against the water
was recorded. Absorbance at 279 nm was measured and the plot of absorbance vs. concentration was plotted.

2.17.1.13 HPLC Method For The Estimation Of Terbutaline Sulfate:

Mobile Phase: Produce a solution consisting of 750 ml of water, 140 ml of methanol, 110 ml of Tetrahydrofuran and 1.08 g of sodium 1-octanesulfonate. Filter and degas. Make adjustments if necessary.

System Suitability Solution: Dissolve appropriate quantities of USP Terbutaline Sulfate RS and 3,5-dihydroxy-6-tert-butylaminoacetophenone Sulfate in water to produce a solution consisting about 50 and 20 µg per ml, respectively.

Standard Preparation: Dissolve an efficiently weighed Quantity of USP Terbutaline sulfate RS in water to produce a solution having a known concentration of about 0.3 mg per ml.

Assay Preparation: Efficiently weigh not less than three containers and separately perform the following procedure for each of the units. Chill in a dry-acetone mixture to about -75 ºC for 15-20 mins. Rapidly and carefully eliminate the top of the container with a tube cutter. Permit the propellant to evaporate at room temperature for 10-15 mins. [Note – avoid complete evaporation of the propellants]. Quantitatively transfer the suspension to a 500 ml separating funnel with the assistance of chloroform. Wash all parts of the container alternately with several small portions of chloroform followed by small portions of 0.01 N sulfuric acid. Transfer the washings to the separatory funnel and adjust the phase volume to about 100 ml each with chloroform and 0.01 N sulfuric acid, respectively. Dry the container and all of its parts at 105 ºC for 1 hour. Cool to room temperature and weigh. Shake the separatory funnel for 1 min, permit the phases to separate, and remove the chloroform layer. Filter the acidic aqueous phase through filter paper into a 250 ml volumetric flask. Wash the separatory funnel with two 10 ml portions of water and transfer the washing to the volumetric flask. Dilute the water to volume, mix and filter, removing the first 2 ml of the filtrate.

Chromatographic system: The liquid chromatograph is equipped with a 280 nm detector and a 6.2 mm × 8 – cm column that contains 3-µm packing L7 and is maintained
at 40 °C, and fitted with a about 0.5-µm pre-column. The flow rate is about 1.5 ml per min. Chromatograph the system suitability solution and recd the peak responses as directed for procedure: the relative retention times are about 0.83 and 1.0 for terbutaline and 3,5-dihydroxy-ó-tert-butylaminoacetophenone, respectively, and the resolution, R, between Terbutaline Sulfate and 3,5-dihydroxy-ó-tert-butylaminoacetophenone is not less than 1.6. Chromatograph the standard preparation and record the peak responses as directed in the procedure: the relative standard deviation for repilicate injections is not more than 2.0%.

**Procedure:** Separately inject equal volumes (about 20 µL) of the Standard preparation and Assay preparation into the chromatograph record the Chromatograms and determine the responses for major peaks. Calculate the quantity, in mg, of \((C_{12}H_{19}NO_{3})_2H_2SO_4\) in each container taken by formula:

\[
250 \left( \frac{r_u}{r_s} \right)
\]

In which C is the concentration, in mg per ml, of USP Terbutaline Sulfate RS in the standard preparation, and \(r_u\) and \(r_s\) are the peak responses obtained from the Assay preparation and standard preparation, respectively.

### 2.17.2 Reviews on Terbutaline Sulfate

1. **Clay MM et al., (1986)** stated that the subjects were studied on four occasions. On three visits they received 2.5 mg terbutaline delivered from three different types of nebuliser, selected on the basis of the size distribution of the aerosols generated; and on a fourth (control) visit no aerosol was given. These results suggest that for β2 agonists small aerosols (MMD < 2 pm) might be advantageous in the treatment of asthma.

2. **Cook RO et al., (2005)** estimated that a novel process for generating sustained release (SR) particles for pulmonary drug delivery is explained. Sustained release of the model drug, terbutaline sulphate (TS), from the microspheres was found to be proportional to drug loading and phospholipid content. Microspheres with a 33% drug loading showed sustained release of 32.7% over 180 min in phosphate buffer. The sustained release microspheres were fabricated as a carrier free dry
powder for inhalation, and shown a favourable Fine Particle Fraction (FPF) of 46.5F1.8% and Mass Median Aerodynamic Diameter (MMAD) of 3.93 ± 0.12 μm.

3. **Joshi MR and Mishra AN., (1999)** studied that In-vitro studies were carried out to understand the comparative drug diffusion pattern, across artificial membrane of the drug and of the prepared liposomes of different liposomal membrane composition. In-vivo studies were undertaken to measure the extent of and time course of pulmonary tissue uptake of administered liposomes consisting terbutaline sulfate on rat lungs. The findings of present estimation predicted that liposomally encapsulated terbutaline sulfate may be utilized for pulmonary drug delivery for maximizing the therapeutic efficacy and decreasing undesirable side effects.

4. **Mathew T and Agrawal S., (2011)** founded that fast melting tablets of Terbutaline Sulphate was formulated by using of superdisintegrants. The tablets were formulated by direct compression technique. Six formulations of tablets were produced containing drug. Prepared tablets were characterized on various parameters. Characterization results exhibits tablet to be within the official limits. Disintegration and wetting time were in limits that are prescribed for mouth dissolving tablets. Dissolution profile of the tablet exhibits that the excipients utilized in the tablet had no negative influence on the release pattern of the drug. It was thus possible to formulate mouth melting tablets of Terbutaline Sulphate using simple and cost effective technique.

5. **R. Chanda et al., (2008)** explained that an oral mucoadhesive controlled delivery system has been formulated for terbutaline sulphate using natural mucoadhesive materials extracted from the edible fruits like Zizyphus mauritiana and Aegle marmelos(Linn.)Cor. that have best mucoadhesive property than synthetic polymer hydroxypropylmethylcelluloseK4M (HPMCK4M). The in vitro mucoadhesive strength and adhesive and swelling property of mucoadhesive materials extracted from the fruits of Aegle marmelos (Linn.) Cor. And Zizyphus mauritiana were characterized and compared with
hydroxypropylmethylcelluloseK4M (HPMCK4M) by Share Stress and Park and Robinson techniques. Various formulations of oral mucoadhesive coated terbutaline sulphate tablets were produced using these natural materials and hydroxypropylmethylcelluloseK4M (H.P.M.C.K4M) and thickness, hardness, weight variation, friability and assay of tablets were tested. The in vitro release of terbutaline sulphate was studied in buffer pH 7.2 at 370C 0.50C The present study exhibits that natural mucoadhesive materials extracted from the fruits of Aegle marmelos (Linn.) Cor. And Zizyphus mauritiana have greater mucoadhesive property than synthetic polymer.

2.17.3 Marketed Formulations of Terbutaline Sulfate

The details of marketed formulations of terbutaline Sulfate is as follows

1. Terbutaline Sulfate 2.5 mg/ml Nebuliser Solution.
2. Terbutaline Sulfate injection
3. Terbutaline Sulfate Syrup
4. Terbutaline sulfate tablets