2 LITERATURE REVIEW

2.1 LUNG CANCER

A risk factor is anything that affects a person's chance of getting a disease such as cancer. Different cancers have different risk factors. Some risk factors, like smoking, can be changed. Others, like a person's age or family history, can't be changed. But having a risk factor, or even several risk factors, does not mean that one will get the disease. And some people who gets the disease may have few or no known risk factors. Several risk factors can make you more likely to develop lung cancer. These risk factors bind with DNA of normal cells and cause mutations which accounts for the pathophysiology of lung cancer (1). Hence, elimination of smoking can be considered as the key factor for the prevention of lung cancer. Avoiding passive smoking is also important as it is equivalently dangerous (2).

2.1.1 Risk factors for lung cancer

If a person has history of lung cancer, he/she may have a higher chance of developing lung cancer again with same or more severity (3). If any family member is diagnosed with cancer at younger age without any contact with direct risk factors, he/she have higher chance of developing cancer due to shared genes. Researchers have found that genetics seems to play a role in some families with a strong history of lung cancer (4). On the basis of its control risk factors can be classified in two categories (5).

2.1.1.1 Risk factors one can control

- Tobacco smoking
- Passive smoking
- Direct radon gas exposure
- Direct exposure from asbestos dust
- Contact with radioactive ores such as uranium
- Inhalation of certain chemicals and heavy metals such as arsenic, beryllium, cadmium, silica, vinyl chloride, nickel compounds, chromium compounds, coal products, mustard gas, and chloromethyl ethers
- Fumes from diesel exhaust
- Impurities of arsenic present in drinking water

2.1.1.2 Risk factors one cannot control

- Former radiation therapy to the lungs
- Air pollution

- Personal or family history of lung cancer

2.1.2 Symptoms of lung cancer

- A chronic cough that worsens with time.
- Coughing up blood i.e. rust-coloured sputum (Haemoptysis)
- Shortness of breath (Dyspnoea)
- Hoarse voice (Dysphonia)
- Difficulty in swallowing (Dysphagia)
- Chronic chest pain which could worsen with deep breathing, coughing or even laughing.
- Drastic weight loss (Cachexia) following loss of appetite
- Restlessness, tiredness
- Recurring lung infections i.e., bronchitis and pneumonia
- Clubbing of fingernails (Rare) (6)

2.1.3 Pathogenesis of lung cancer

Very similar to other cancers, lung cancer also occur due to activation of proto oncogenes (7) and subsequent process which is shown in Figure 2-1.

Mutations occur in K-RAS type of proto-oncogene which are solely responsible for the lung adenocarcinoma (10-30%). Another mutation in EGFR is responsible for the occurrence of non-small cell lung cancer as EGFR plays significant role in the process i.e., proliferation, apoptosis and angiogenesis and even invasion of tumor. Heterozygosity produced by damage in chromosomes are also responsible for the suppression of the tumor suppressor gene. In most studies p53 tumor suppressor gene, which is located at 17 base pair of chromosome has been considered responsible for 60-75% of the registered NSCLC cases worldwide (8).

Genetic polymorphism associated with gene coding for the CYP-450 and capase-8 (apoptosis promoters) are also associated with lung cancer. Person possessing these types of polymorphism in genes have high chance of developing lung cancer after exposure to carcinogens.

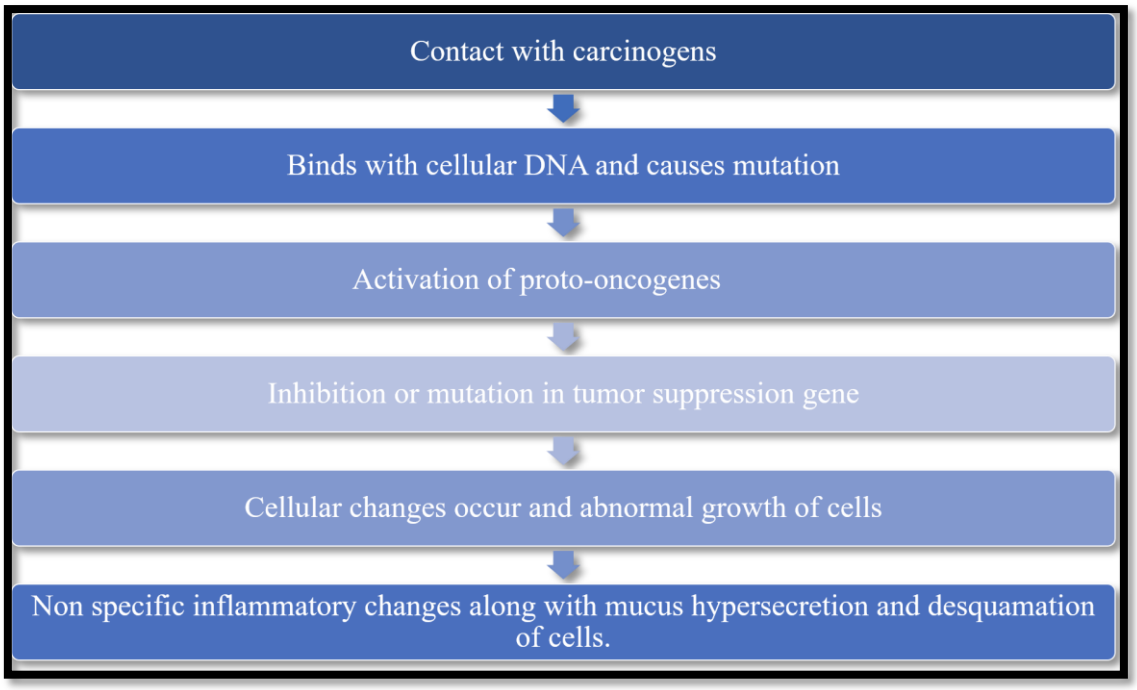


Figure 2-1 Pathogenesis of lung cancer

2.1.4 Prevention and treatment

Smoking cessation and reducing exposure to carcinogens are the primary and only tools for the prevention of lung cancer.

2.1.4.1 Surgery

PET (Positron Emission Tomography) has been used to determine level of disease i.e., localized which is permissible for surgery and/or it has spread to utmost point where surgery cannot be a possible cure. VATS (Video-assisted thoracoscopic surgery) lobectomy has been approached for least invasive surgical approach for lung cancer with advantages of speedy recovery (9). Types of surgery has been listed here.

- Pneumonectomy
- Lobectomy
- Segmentectomy or wedge resection
- Sleeve resection

2.1.4.2 Radiofrequency ablation (RFA)

RFA technique uses high-energy radio waves to heat the tumor. A thin, needle-like probe is pierced through the skin and is inserted until the tip is in the tumor. Placement of the probe is guided by CT scans. Once the tip is in place, an electric current is passed through the probe, which heats the tumor and destroys the cancer cells (10).

2.1.4.3 Radiation therapy

It is mostly combined with chemotherapy and preferred for the patient to whom surgery is not the cure. It is a **'continuous hyper fractionated accelerated radiotherapy (CHART)'** technique, in which a high dose of high energy X-rays are given for a short period of time (10).

- Radiation or radical therapy generally uses high-energy X- rays to kill cancer cells. Mainly two types of radical therapy are present which are listed here: External beam radiation therapy and internal radiation therapy
- Brachytherapy (internal radiation therapy)
  - Given inside the airway when cancer affects a short section of bronchus. It is used when inoperable lung cancer causes blockage of a large airway.

2.1.4.4 Chemotherapy

The chemo drugs most often used for NSCLC include (11):

- Gemcitabine (Gemzar®)
- Cisplatin
- Carboplatin
- Docetaxel (Taxotere®)
- Paclitaxel (Taxol®)
- Albumin-bound paclitaxel (nab-paclitaxel, Abraxane®)
- Vinorelbine (Navelbine®)
- Irinotecan (Camptosar®)
- Etoposide (VP-16)
- Vinblastine
- Pemetrexed (Alimta®)

2.1.4.5 Targeted therapies

✓ **Drugs that target cells with EGFR mutations(12)**

Epidermal growth factor receptor (EGFR) is a protein on the surface of cells. It normally helps the cells to grow and divide. Some NSCLC cells have too much EGFR, which makes them grow faster. Drugs called *EGFR inhibitors* can block the signal from EGFR that tells the cells to replicate/multiply. Some of these drugs can be used to treat NSCLC (13).

a. EGFR inhibitors used in NSCLC with *EGFR* gene mutations

- Erlotinib (Tarceva®)
- Afatinib (Gilotrif®)
- Gefitinib (Iressa®)

b. EGFR inhibitors that also target cells with the T790M mutation

- Osimertinib (Tagrisso®)

c. EGFR inhibitors used for squamous cell NSCLC

- Necitumumab (Portrazza®) (MoAb)

✓ **Drugs that target cells with ALK gene changes**

Anaplastic lymphoma kinase (ALK) is an enzyme that assists the cell growth and division. It is regulated by the ALK gene. There is a modification (mutation) in the ALK gene in a relatively limited number of non-small cell lung cancers, which allows the cancer cells to expand and spread. ALK mutation are considered in cancer cells that have ALK positive (ALK+).

- Crizotinib (Xalkori®)
- Ceritinib (Zykadia®)
- Alectinib (Alecensa®)

✓ **Drugs that target tumor blood vessel growth (angiogenesis)**

- Bevacizumab (Avastin®)
- Ramucirumab (Cyramza®)

2.1.4.6 Immunotherapy

Immunotherapy has been used as adjuvant therapy which acts by increasing one's own immune system that can be helpful in destroying cancer cells. PD-1 is a protein present on T-cells which helps to prevent cancer by attacking cancer cells. Nivolumab (Opdivo®) and pembrolizumab (Keytruda®) are the only available drug that targets PD-1 and blocks its preventive action on tumor cells which results in reduction of the tumor bulk and slows down its growth (14).

2.1.4.7 Palliative treatment

Palliative treatment aims to manage symptoms without trying to cure the disease. It can be used at any stage of lung cancer to improve quality of life. Palliative treatment is about living for as long as possible in the most satisfying way one can (15). Following are few drugs helpful to manage cancer:

➢ **<u>Treating fluid build-up in the area around the lung</u>**

- Thoracentesis
- Pleurodesis
- Catheter placement

➢ **<u>Treating fluid build-up around the heart</u>**

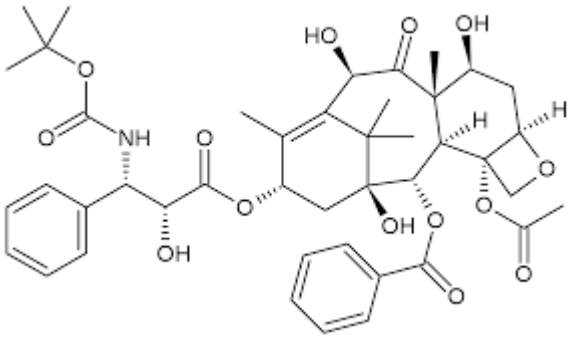
- Pericardiocentesis

➢ **Treating an airway blocked due to tumor**

- Photodynamic therapy (PDT)
- Laser therapy
- Stent placement

2.2 DRUG PROFILE

Table 2-1 Docetaxel profile

Drug	Docetaxel
Molecular formula	$C_{43}H_{53}NO_{14}$
Molecular weight	807.89 g/ mol
Molecular structure	
Bioavailability	Orally: 8±0.6% ,IV : 100 %
Log P	4.26
BCS class	Class IV
Docetaxel as single agent	75 mg/m^2 single agent after platinum therapy failure administer intravenously over 1 hr every 3 weeks.
pKa	10.96 (Strongest Acidic)
$t_{1/2}$	The half-life of the alpha, beta, and gamma phase are 4 minutes, 36 minutes, and 11.1 hours, respectively.
Solubility	Soluble in DMSO (5 mg/mL), Ethanol (1.5 mg/mL) and DMF (5 mg/mL) at 25°C, Methanol(2mg/mL)
Absorption	The pharmacokinetic profile is consistent with a three-compartment model.
Elimination	Docetaxel was eliminated in both urine (6%) and faeces (75%)
Melting point	179 °C

Adverse Drug Reactions	Hepatotoxicity, Neutropenia, Hypersensitivity reaction and Fluid retention.

2.2.1 Mechanism of action

Docetaxel is a well-known antineoplastic drug and it acts by disrupting the microtubular network in cells that is essential for vital mitotic and interphase cellular functions. Docetaxel promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. Docetaxel binds to free tubulin thereby decreasing the critical intracellular concentration of tubulin. The promoted polymerization of microtubules leads to the production of microtubule bundles without normal function and to the stabilize microtubules, resulting in the inhibition of mitosis in cells (16). The binding of Docetaxel to microtubules does not alter the number of proto filaments in the bound microtubules; in fact, it differs from other spindle poisons. Docetaxel was found to be cytotoxic in in vitro against various murine and human tumor cell lines, and against freshly excised human tumor cells in clonogenic assays. In addition, Docetaxel was found to be active on a number of cell lines overexpressing the p-glycoprotein, which is encoded by the multidrug resistant gene (17). Figure 2-2 shows demographic representation on mechanism action of Docetaxel.

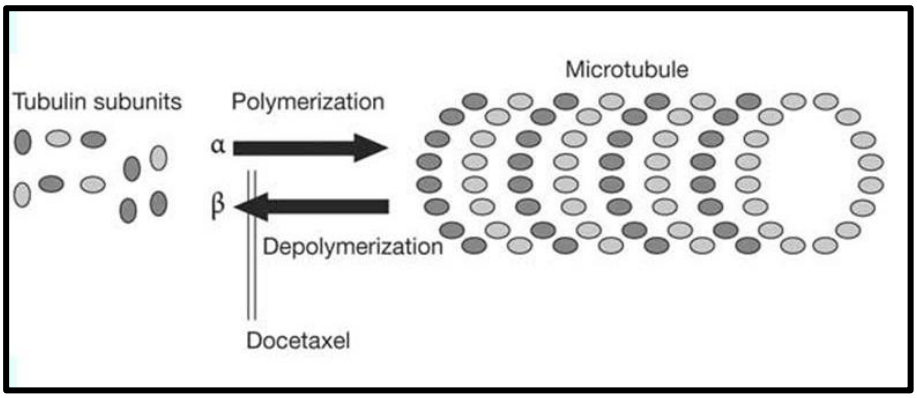


Figure 2-2 Demographic representation of Docetaxel mechanism of action

2.2.2 Problems associated with marketed formulation

Docetaxel is practically insoluble in water. Hence, marketed formulation of Docetaxel (Taxotere®) is formulated using non-ionic surfactant and ethanol to enhance its solubility. Incorporation of polysorbate 80 may lead to hypersensitivity reactions that occurs very commonly during infusion and it affects almost 25-30% of patients (18). As a general practice to prevent the hypersensitivity reaction glucocorticoids (mostly Dexamethasone) premedication are given to patients and has become a standard practice. The side effects of dexamethasone and polysorbate 80 cumulatively lead to treatment related morbidity and in

some cases, it causes early discontinuation of the therapy. Along with that polysorbate 80 may cause peripheral neuropathy (chronic side effect of Docetaxel). With addition, polysorbate 80 and ethanol may leach plasticizer from the infusion sets and PVC bags of saline during infusion which accounts for worsen side effects.

2.2.3 Resistance to Docetaxel

Multidrug resistance (MDR) is the most frequent phenomenon by which cancer cells elude chemotherapy. Mechanisms responsible for the MDR can be broadly divided into cellular factors and physiological factors. Multidrug resistance (MDR) is the most frequent phenomenon by which cancer cells elude chemotherapy. Cellular factors include altered molecular targets, increased drug metabolism, genetic defects such as polymorphism and gene deletion, reduced apoptosis, and over-expression of efflux pumps (19)whereas physiological factors include cell–cell interaction, higher interstitial fluid pressure, low pH environment, hypoxic region in the tumor core, irregular tumor vasculature, and the presence of cancer cells in areas difficult to penetrate (20). Most of these factors lead to the requirement of higher doses of chemotherapeutic agents, which demonstrate systemic toxicity. Based on the type of disease and given treatment, mechanisms responsible for the demonstration of MDR vary.

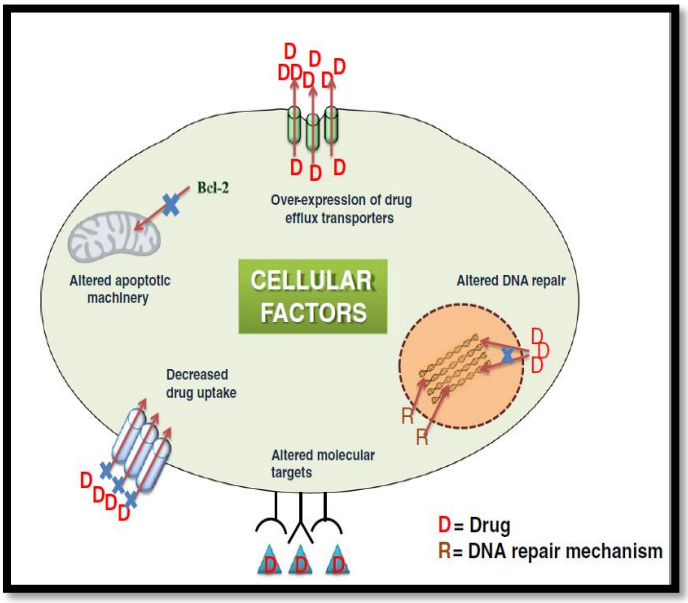


Figure 2-3 Cellular factors responsible for drug resistance

These factors are inter-related and considered to affect one another.

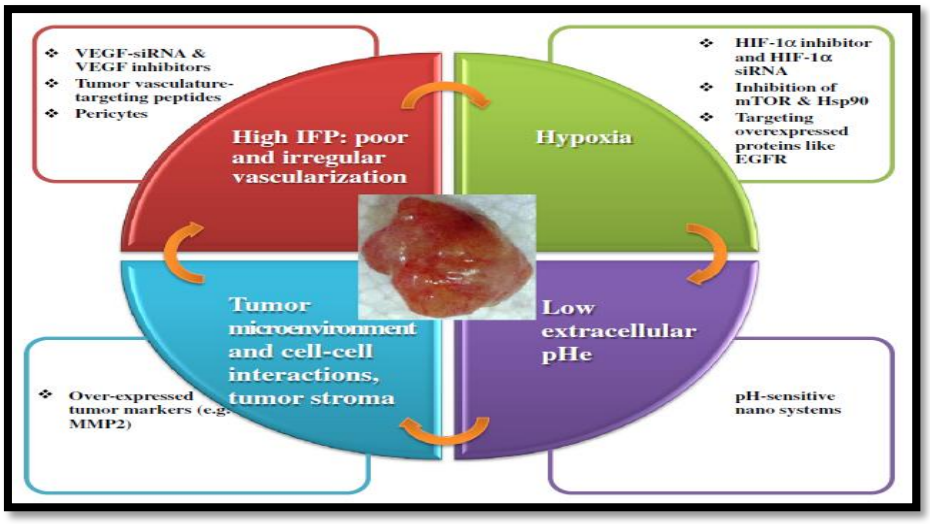


Figure 2-4 Physiological factors responsible for drug resistance

The emergence of resistance to the administered medication and chances of tumor developing refractoriness is a prominent issue. Combination therapy offers stimulus to pursue research for effectively treating multi drug resistant (MDR) lung cancer. It is well established that ATP-binding cassette (ABC) transporters like MDR1 (ABCB1), MRP1 (ABCC1) and ABCG2 etc. are responsible for the efflux of anti-neoplastic drugs from cancer cells including lung cancer (21). The success rate of chemotherapy and survival of patient correlate with expression of these transporters. The chemical inhibitors of ABC transporters have very low success rate so there is ample motivation to develop strategies which may prevent induction of ABC transporter in cancer cells that may avert drug resistance. P-gp, a member of the ABCB subfamily, positions out among ABC transporters by conferring the strongest resistance to several anti-neoplastic agents (22). P-gp is a glycoprotein that in humans is encoded by the ABCB1 gene. It is reported that MDR-1 is responsible for conferring resistance to lung cancer cell lines hence down -regulation of ABCB1 genes specific to cancer cells in lung cancer makes it more appropriate target.

Table 2-2 Approaches used in the reversal of drug resistant cancer.

Vector	Mode of reversal of resistance	Cytotoxic agent	Tumor type	Remarks
Nano constructs (23)	Anti-sense ODNs targeted to BCl 2 &MRP1 mRNA	Doxorubicin	Lung cancer	Showed high antitumor activity and low adverse side effects of proposed complex inhalatory treatment that cannot be achieved by individual components

				applied separately. The present work potentially contributes to the treatment of lung cancer by describing a unique combinatorial local inhalation delivery of drugs and suppressors of pump and non-pump cellular resistance.
Magnetic Fe_3O_4 nanoparticles (24)	Combination therapy with MDR1 shRNA	Daunorubicin	Leukemia	Combination of DNR with either MNP (Fe(3)O(4)) or PGY1-2 exerted a potent cytotoxic effect on K562/A02 cells, while combination of MNP (Fe(3)O(4)) and PGY1-2 could synergistically reverse multidrug resistance.
Poly(n-butylcyano acrylate)Nanoparticles (25)	Inhibition of P-gp function	Paclitaxel	Ovarian cancer	Paclitaxel-loaded PBCA nanoparticles can enhance cytotoxicity and overcome MDR through a mechanism of the inhibition of P-gp function caused by the nanoparticles system.
Multi-functional nano-carrier (26)	siRNA targeted to BCl 2 & MRP1 Mrna	Doxorubicin	Lung cancer	It enhanced efficiency of chemotherapy to a level that cannot be achieved by applying its components separately
PLGA nanoparticle (27)	siRNA-Stat3	Paclitaxel	Lung cancer	These nanoparticles suppressed Stat3 expression and induced cellular apoptosis in human lung cancer cell lines (A549)

2.3 GENE DELIVERY

The fundamental hereditary causes of a variety of modern human illnesses, and pharmacological treatments frequently fall short of treating most of these diseases. Gene therapy is characterized by presenting the missed part as a means of permanently treating or restoring certain faulty or deleted genes (28). Gene therapy represents a novel approach that can be used in both genetic and inherited disease care. Gene therapy pioneers have used methods of modifying faulty genes, such as replacing a non-functional gene with regular gene

or an abnormal gene with a normal gene through recombination, or fixing an irregular gene through selective reverse mutations by selectively regulating the expression of the mutated gene. The genes can also be administered as genetic vaccines in another form of application to cause both cell-mediated and humoral immune responses (29).

2.3.1 RNAi mechanism

RNA interference (RNAi) is a biological process by which the degradation of complementary mRNA and the sequence-specific inhibition of a particular gene is driven by a small dsRNA. Many disorders are targeted by complex gene expression/ repression after transcriptional silencing. These functions were identified and formally published in Nature in 1998 (30), but when Andrew Fire and Craig Mello won the Nobel Prize in physiology and medicine in 2004, it gathered momentum. During their gene expression regulation studies, they studied the RNAi phenomenon in the nematode worm Caenorhabditis elegans, which is explained in Figure 2-5. The easy synthesis of siRNA makes RNAi technology fast and effective. This is because there is no cellular expression system prerequisites, steps and sequences related to upstream and downstream protein purification can be built from sense and antisense strand of target-related particular genetic significance knockdown. Compared to siRNA, the distribution of shRNA pDNA is a simple and efficient process. This is because the cytosol is the site of action for siRNA, while shRNA plasmids and pDNA have to join the nucleus for their action hence provide sustained effect (31).

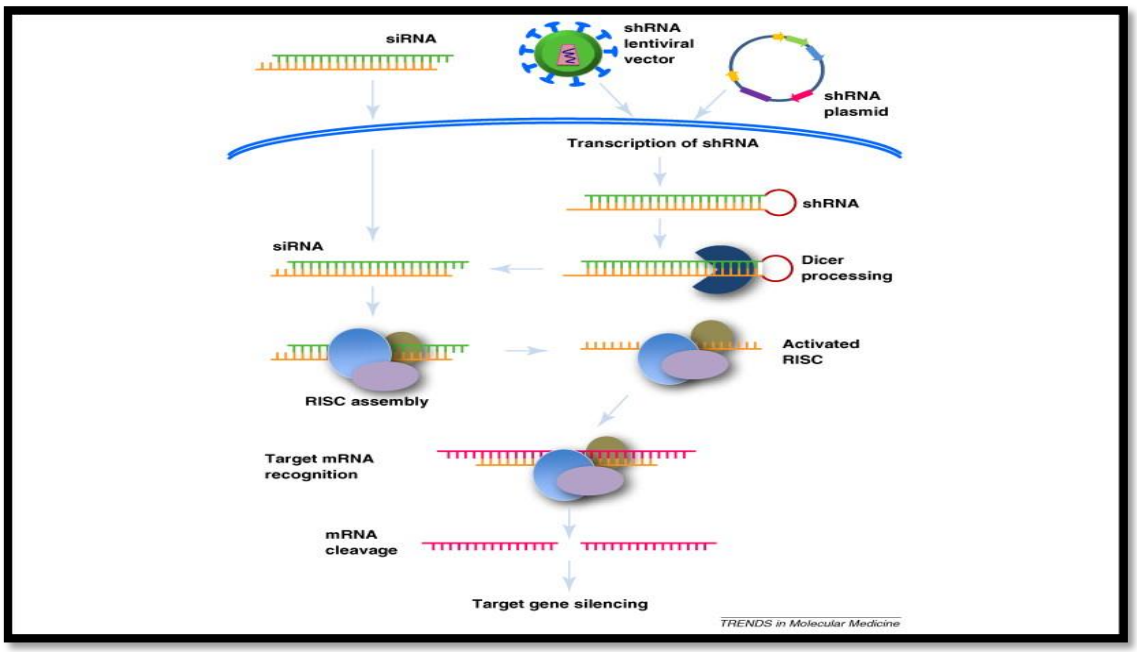


Figure 2-5 Gene silencing mechanism through RNA interference technology (RNAi) (32)

2.3.2 shRNA

shRNA is short hairpin RNA, double-stranded RNA (dsRNA) that is formed from a DNA build in the cell encoding a single-stranded RNA string and its counterpart, separated by a stuffer fragment, enabling the RNA molecule to coil back on itself, forming a hairpin loop (33). In its synthetic version, shRNA suffers from the same limitations as siRNA mentioned above. However, if it is derived from a DNA model within the cell, it has the positive attributes compared to siRNA, hence it is continually produced by the process of the target cell itself by the cassette of genes that travels to the nucleus. The added DNA here either becomes part of the cell's own DNA or remains in the nucleus and instructs the cell to generate the particular shRNA that Dicer the processes to siRNA and continues through RISC to silence the gene along the RNAi pathway. Unlike siRNA, a small dose of DNA (in the range of 5 copies) is needed for DNA-directed shRNA process, hence the effect is long-lasting from a single dose of small quantity shRNA plasmid. (Shown in Figure 2-6 (34).

Several different vector expression systems have been reported. Scherer et al. have created tRNA(Lys3)-shRNA chimeric expression cassettes that produce siRNAs with similar efficacy and strand selectivity to those of U6-expressed shRNAs, and their activity is consistent with processing by endogenous 3′ tRNAse (35). Results reported by Takahashi et al. have shown that the U6 promoter has more stable efficiency than H1 or tRNA promoters in driving shRNA vectors (36). However, another research group has demonstrated that a modified tRNA(met)-derived (MTD) promoter effectively drives the cellular expression of HIV-1-specific siRNA, and shows greater inhibition of virus production when the MTD promoter is used rather than cassettes that contain other polymerase III promoters, such as H1 and U6 (37).

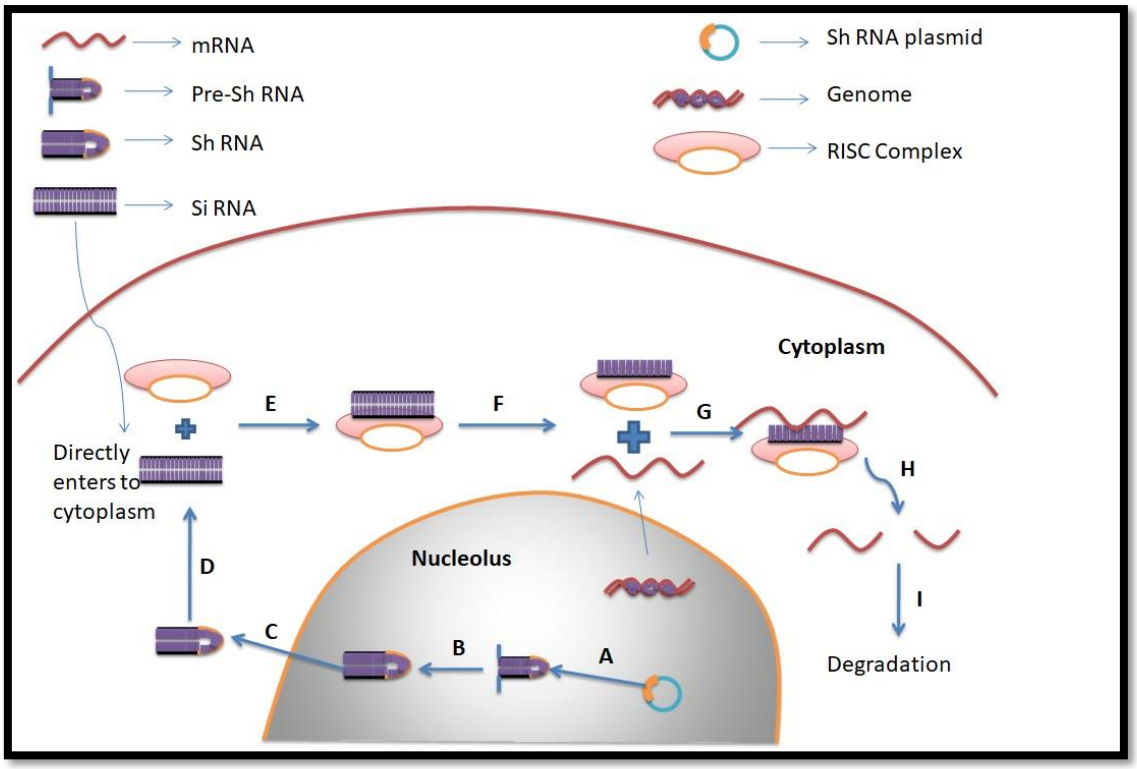


Figure 2-6 Difference between gene silencing effect of siRNA and shRNA

2.3.3 In Vivo Delivery Vectors

Efficient aggregation of RNA macromolecule intracellularly is most troublesome in the distribution of RNAi. Various methods, including viral and non-viral transmission of vectors, local and structural route administration, have been used to control target protein down regulation. To induce the RNAi reaction in vivo, various formulations varying from saline solution of naked shRNA to lipid, protein or conjugates, aptamers etc. have been used (38).

To use them in clinical application, each of these have distinct merits and demerits. For several factors, RNAi molecules needs a delivery vector. This involves elevated negative charge, molecular weight and nuclease degradation. When transfection efficiency is in doubt, viral vectors are more advantageous. However there are major benefits of non-viral vectors, i.e. less in-vivo toxicity, immunogenicity and insertional mutagenesis (39). An ideal RNAi delivery vector should be fitted with an appropriate transfection cationic group, an endosomal escape endosomolytic group, a surface modifier to decrease steric disability that eventually improves blood circulation time, and a targeting moiety to guide the delivery mechanism to target cells or tissue. With respect to systemic transmission, the scale of delivery vectors in the biological system plays an enormous role. The duration of the distribution vector should not be less than 5 nm to prevent glomerular filtration. At the same time, the distribution mechanism

should be sufficiently wide to prevent leakage of hepatic sinusoid interstitial spaces and trapping of hepatic Kupffer cells that need a particle size greater than 100 nm. In addition, the scale should not be more than 200 nm to inhibit macrophage absorption into systemic circulation (40). Therefore, the optimum distribution vector size should be between 100-200 nm for systemic delivery.

Viral vectors normally induce long-term target protein inhibition in a single administration, but these vectors are subjected to substantial risk from host immune response, which has recently been highlighted. Diversion of the RNAi system, which eventually manifests in marked toxicity, was observed earlier with the use of adeno-associated virus mediated shRNA delivery in mice (41). Non-viral transport vectors have been used widely, locally and systemically, to transmit nucleic acids. This specifically contain lipids, peptides, and polymers. For the delivery of RNAi to the target site, various lipid complexes, liposomes, polymers, proteins and antibodies have been used. There are some cytotoxic effects of cationic lipids and polymers that can hinder the use of these carriers for specific disease indications and dosing paradigms in RNAi delivery (38). In the end, a carrier device that has the least in-vivo toxicity with increased transfection efficacy should be progressed.

Oligofectamin® , Lipofectamine®, TransIT-TKO® and DharmFECT®, all of which have been used to transport RNAi macromolecules in vivo, are some of the widely active lipid-based non-viral RNAi vectors for transfection (42). Positively charged lipids are usually used for RNAi complexation and transmission, but few of the neutral and anionic lipids have also been tested. According to few studies cationic lipids have low cytotoxicity stability and reproducibility in in vivo. Improvement of the charge ratio between vector and amino acid is important for efficient distribution of RNAi, with minimal side effects, since it is the negative charge of the RNAi molecule that complexes with the cationic vector group (43). Normally, cationic lipids or liposomes composed of these lipids are much more harmful than their neutral counterpart.

Polymer vectors provide a lower immune response compared to lipid-based vectors, but not safer in the unmodified form. Polymers have consistency for use in terms of their physical and chemical properties, which may be the key source of detailed studies on RNAi therapeutic polymer distribution. The complex formation of polycations and RNAi, also known as polyplexes, is greatly influenced by charge density, molecular weight, and pH. In comparison to the DNA molecule, polycations interact weakly with RNAi and thus eventually contribute to loosen polyplex formation. Increased charge ratio can mitigate this downside, but eventually

increased charge density results in a reduced protection margin with regards to cytotoxicity (44).

Cationic polymer Polyethyleneimine (PEI) is one of the most widely researched non-viral delivery vectors for endothelial transfer of RNAi to local and structural applications. PEI has also been used, as a standard reference for many in-vitro and in-vivo tests. This cationic polymer's proton sponge effect results in endosomal release of RNAi into the cytoplasm and ensures high efficiency of RNAi transport (44). However, for secure and convenient RNAi delivery, in-vivo sensitivity of PEI has prompted researchers to create new adapted polymers and polycations. In addition, one of the alternative means of delivering RNAi is nanoparticles made up of hydrophobic polymeric matrix encapsulating macromolecule RNAi.

2.4 POLYMER LIPID HYBRID NANOCARRIERS (PLHNC)

Nanomedicines has been attracted many researchers because its ability to delivery therapeutic drugs at particular target with good efficiency. Hence therapeutic level of the drugs has been achieved in a more precise manner for longer period of time. Among all the nanocarriers liposomes and polymeric nanoparticles have been most widely researched as novel strategy for delivery of variety of therapeutics including genetic materials due to their biocompatibility and in-vivo drug targeting. Hence both nanocarriers possess few disadvantages, it is prerequisite to superimpose these disadvantages with each other's advantage. Polymeric nanoparticles are a class of artificial vesicles which is made from synthetic amphiphilic block copolymers. The key attributes of these membrane systems are their long- term stability and tunability by varying the chemical makeup of their copolymers. The selection of copolymer's molecular weight controls the membrane thickness and hence the permeability and mechanical properties of the membrane but are generally lacking in inherent biocompatibility and potential toxicity of long-term accumulation of synthetic molecules in the body (45). The liposome is a closed vesicle composed of a lipid bilayer membrane and readily used for the functional reconstitution of integral membrane proteins. The biocompatible, non-denaturing interface of liposomal capsules also makes this a suitable material for encapsulation and isolation of biomolecules (46). Unfortunately, the lack of long-term stability of liposomes can be problematic for applications which require extended shelf lives or prolonged monitoring times. In addition, membrane specific interactions with various molecules in the host media can acutely perturb the structural integrity of liposomes so there is an ample room to develop such a hybrid delivery system which bring together the excellent mechanistic stability and chemical tunability of polymeric nanoparticles with inherent biocompatibility and bio-

functionality of liposomes (47, 48). The present proposal focuses to prepare a novel nano-carrier cargo PLHNCs and evaluate its properties to maximize transfection process.

2.4.1 Types of lipid polymeric hybrid vesicles

Table 2-3 Types of lipid polymeric hybrid nanoparticles

Type	Description	Synonyms
Polymer core–lipid shell	Colloidal supramolecular assemblies consisting of polymer particles coated with lipid layer (s)	Lipoparticles Lipid–polymer particle assemblies Lipid-coated NPs Nanocell Polymer-supported lipid shells
Core–shell-type hollow lipid–polymer–lipid NPs	Hollow inner core surrounded by concentric lipid layer, followed by polymeric layer, again followed by lipid layer along with lipid–PEG.	
Erythrocyte membrane-camouflaged polymeric NPs	Sub-100-nm polymeric particles are coated with RBC membrane derived vesicles to mimic complex surface chemistry of erythrocyte membrane	Biomimetic NPs
Monolithic LPHNs	Lipid molecules are dispersed in a polymeric matrix	Mixed lipid–polymer Particles
Polymer-caged liposomes	These systems are composed of polymers, anchored or grafted at the surfaces of the liposomes to provide stability	

2.4.2 Advantage of PLHNCs

PLHNCs has advantages of both liposomes and polymeric nanoparticles. Some extraordinary advantage performed by PLHNCs are listed here.

- The solid core made up of polymer acts as a cytoskeleton that provides mechanical stability, controlled released morphology, narrow size distribution, and higher availability of specific surface area.
- The outer lipid coat that encapsulates the polymeric core is biocompatible in nature and mimics the characteristic of cellular membranes. The lipid shell can interact with a huge variety drugs and indigenous molecules and surface can be modified for efficient targeting.
- Improved encapsulation of hydrophobic drugs with effective drug entrapment efficiency and drug loading has been reported for a number of drugs compared to liposomes or polymeric nanoparticles.
- Amphiphilic character of lipids facilitates the adsorption of hydrophilic compounds on the bilayer surface and insertion of hydrophobic molecules into the hydrophobic lamellar region. This feature allows PLHNCs to entrap and deliver multiple hydrophilic and hydrophobic therapeutic agents simultaneously.
- Optimization of the core and shell can result in tunable and sustained drug release profiles.
- PLHNCs exhibit storage and serum stability over prolong period.
- Besides passive targeting of PLHNCs based on particle size, they can be conjugated with appropriate targeting ligands such as aptamers, folic acid, transferrin, anti-carcinogenic embryonic antigen half-antibody, or single chain tumor necrosis factor to deliver PLHNCs at the target tissues for treating cancers.
- Particles smaller than 100 nm (similar to virus-like architecture) are promising for intracellular drug targeting and vaccine adjuvants.

2.4.3 Methods for preparation of PLHNCs

The Methods used to prepare PLHNCs broadly fall into two categories; the two-step method and the single-step method.

2.4.3.1 Two-Step method

Polymer core is prepared separately using methods used to prepare polymeric nanoparticles and lipid layer is prepared separately mimicking liposome preparations. Then

two different assemblies are mixed up using process i.e. Ultrasonication, direct hydration, homogenization and extrusion to obtained desired sized polymeric core-lipid shell nanocarriers (49, 50). Demographic representation of the two-step method is shown in Figure 2-7.

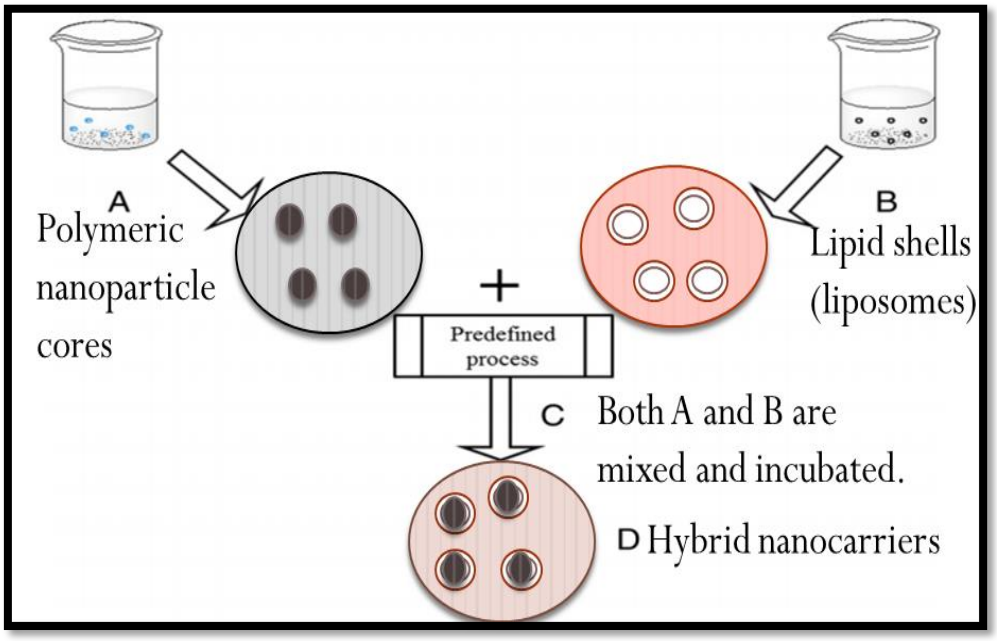


Figure 2-7 Two-step method

2.4.3.2 Single step method

Single step method is relatively more used as it combines both step at the same time. It is work on the principle of the self-assembly. Single step method is further divided in to two approaches.

2.4.3.2.1 Modified solvent evaporation method

Briefly, the block copolymer along with hydrophobic drug have been dissolved in immiscible mixture of water with any organic solvent i.e., DCM (dichloromethane), chloroform, or even ethyl acetate. A fixed quantity of lipid has been dispersed in double distilled water with help of bath sonication, heat and/or mechanical stirring. Then organic phase has been mixed with aqueous phase with continuous stirring. Tiny droplets formed of the organic phase has been solidifies in the form of nanoparticles which has outer coating of lipid membrane. Extra organic solvent is then evaporated by overnight stirring or by incubating in vacuum desiccator (51, 52).

2.4.3.2.2 Single step nano-precipitation

In single step nano-precipitation, polymer along with hydrophobic drug(s) have been solubilized in water miscible solvent i.e., ACN (acetonitrile) or Acetone. Then organic phase was added drop by drop in a controlled manner to aqueous phase containing lipid or

combination of lipids. The resulting mixture was vortexed and homogenised or ultrasonicated to achieve desired particle size, most probably in nanoscale (52).

The basic difference in both single step methods has been demonstrated in the Figure 2-8.

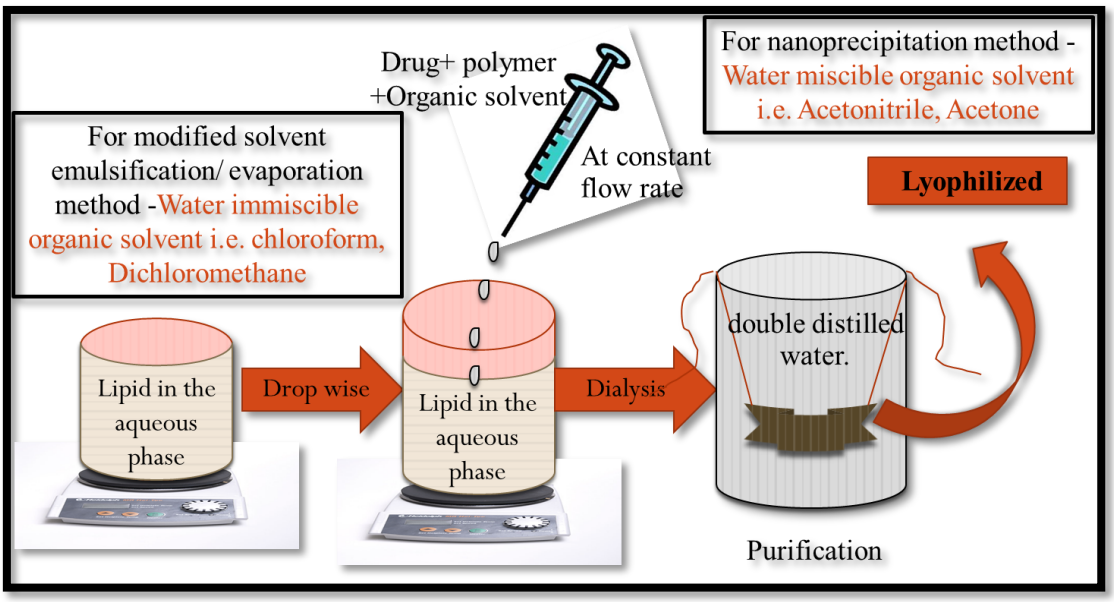


Figure 2-8 Single step method

2.4.4 Recent literature on the Hybrid nanocarriers

Table 2-4 Recent literature on development of hybrid nanocarriers

Encapsulant	Polymer	Lipid	Particle size	EE/DL	Application	Reference
Doxorubicin and combretastatin	PLGA	PC/Chol/DSPE-PEG	180-200 nm	NR	Melanoma , Lewis lung carcinoma	(53)

Doxorubicin	PLGA	DEPE-PEG Lecithin	118.7 ± 0.75	45.76 ± 6.58	Folate receptor mediated drug delivery of anti-cancer agent, doxorubicin, resulted in higher cell internalization and enhanced cell-killing effect toward MCF-7 cells with a significantly lower IC50.	(54)
Docetaxel	PLGA	Lecithin PEG	70–80	59 ± 4	Docetaxel loaded Hybrid nanoparticle exhibited 20 hourrs as T_{50}. These carriers also exhibited good stability in 10% bovine serum albumin and in 10% plasma solution.	(55)
Paclitaxel	PLGA	Soybean lecithin D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)	120–150	>80	Developed carriers provided sustained release up to 8 days with a high tumor targeting potential through EPR effect. It also showed superior antitumor efficacy by inhibiting 58.8% volume of tumor at day 28.	(56)

Docetaxel	PLGA	DEPE-PEG$_{2000}$	263.6	66.88	Folic acid conjugation increased 38.2% for 0.5 hour incubation and 54% increase for 2 hours incubation during cell uptake study. Cell viability studies showed that formulation was 93.65% more effective than commercial preparation Taxotere®.	(57)
Paclitaxel	Poly-lactic-co-glycolic acid (PLGA	Soybean lecithin 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG)	186.9 ± 8.52	81.34 ± 3.41	More drug reaches target site crossing Blood brain barrier and survival time for mice was PtxR-FPLNs (42 days), Ptx-FPLNs (38 days) compared to PtxR (18 days) and Paclitaxal (14 days)	(58)
Melatonin)	Poly lactic acid (PLA)	Didodecyldimethylammonium bromide (DDAB) Cetyltrimethylammonium bromide (CTAB	180–218	90.35	Coating with cationic lipids provides sustained and prolonged drug release, a pronounced benefit in ophthalmic application	(59)

Curcumin	PLGA	DPPC DSPE-PEG	171.6 ± 8.2	NA	By treating the metastatic breast cancer cells with the lipid-polymer hybrid nanoparticles of Curcumin decreased the adhesion onto tumor necrosis factor by 70% in capillary flow.	(60)
Docetaxel	PLA	Chitosan	208–255.7	75.9	PLA/chitosan nanoparticles provide rapid initial release of 40% drug in 5 hours and 70% cumulative release in 24 hours.	(61)
Doxorubicin	Chitosan	Hyaluronic acid	264 ± 2.2	97.8 ± 1.3	It is used to deliver anticancer drugs which results in enhanced circulation half-life and reduce the elimination of drug	(62)
Docetaxel	PLGA	DSPE-PEG	110 ± 13.5	77.65 ± 0.57	The system increases the cellular update of docetaxel 2.5 folds and anti-proliferative activity 2.69–4.23 folds.	(63)

Erlotinib	PLGA	DEPE-PEG_{2000} Dipalmitoyl phosphatidyl choline (DPPC) N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylam monium methyl-sulfate (DOTAP)	161–271	77.18	Erlotinib loaded Core Shell Lipid Polymer Hybrid Nanoparticles demonstrated 170 nm size with 66% Entrapment efficiency and greater uptake and efficiency in A549 cells.	(64)

Abbreviations: EE: Entrapment Efficiency; DL: Drug Loading; NR: Not Reported; HPESO: Hydrolyzed Polymer of Epoxidized sSybean Oil; MDR: Multi-Drug Resistant; PLGA: Poly(Lactic-co-Glycolic Acid); DLPC: Dilinoleoylphosphatidylcholine; DMPE-DTPA: 1,2-ditetradecanoyl-sn-glycero-3-phosphoethanolamine-N-diethylenetriaminepentaacetic acid;DSPE-PEG,1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol); PMOXA-PDMSPMOXA,poly(2-methyloxazoline)-block-poly(dimethylsiloxan)-block-poly(2-methyloxazoline);DPPC, dipalmitoylphosphatidylcholine; PEI, polyethyleneimine; EPC, 1,2-dimyristoleoyl-sn-glycero-3-ethylphosphocholine; PGA, poly(glutamic acid); DPTAP, 1,2-dipalmitoyl-3-trimethylammonium-propane; PLA, poly(lactic acid); OQLCS, octadecyl-quaternized lysine-modified chitosan; DHA, cis-4,7,10,13,16,19-docosahexanoic acid; PBAE, poly-(β-amino ester).

2.5 FORMULATION OPTIMIZATION

There are several steps for optimization of drug delivery systems (DDS) with design of experiment (DoE). Broadly, in seven salient phases, these stages can be summarized sequentially (65).

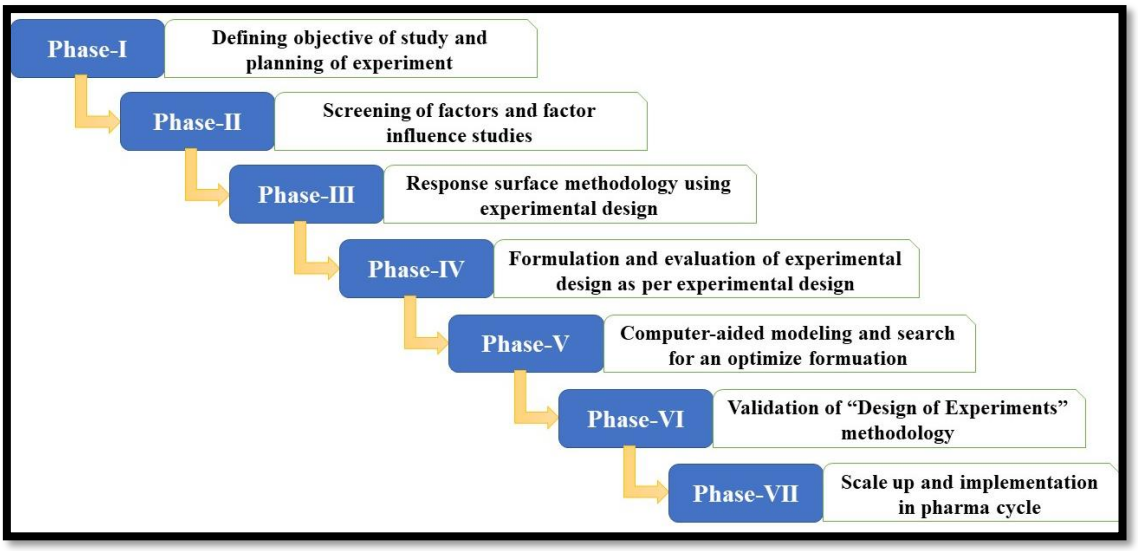


Figure 2-9 Seven-phase ladder for optimizing drug delivery systems

2.5.1 Experimental Designs

The twin basic features of the general scientific approach are the execution of the experiment and the resulting analysis of its experimental outcome (66). Only if the experiments are carried out in a systematic manner, the inferences of the change are predictable. Numerous forms of experimental designs exist. Various widely employed experimental designs for RSM, sampling, and factor-influence trials in pharmaceutical product developement area.

I. Factorial designs
II. Fractional factorial designs
III. Plackett-Burman designs
IV. Star designs
V. Central composite designs
VI. Box-Behnken designs
VII. Center of gravity designs
VIII. Equi-radial designs
IX. Mixture designs
X. Taguchi designs
XI. Optimal designs
XII. Recht-schaffner designs
XIII. Cotter designs

For three-factor analysis, an experimental architecture can inevitably be envisaged as a "cube," with the potential configurations of the factor levels (low or high) represented at its

respective corners (67). Therefore, the cube could be the most fitting depiction of the experimental area being studied. Therefore, most forms discussed here are pictorially represented using this cubic model, with experimental points at the edges, centers of faces, edge centers, and so on. Such representation makes it easier to grasp different styles and similarities between them. The same definition is true to designs in which more than three parameters are modified, so that the hypercube reflects the experimental area. These cubic designs are common because to conceptualize and visualize the model, they are symmetrical and straightforward.

2.5.2 Design Augmentation

In the whole DoE , a condition often exists in which an analysis performed at some point is considered to be insufficient and has to be further studied, or where it is appropriate to reuse the study carried out during the initial stages (68). It is possible to upgrade the former primitive design to a more modern design that offers more details, improved reliability and higher resolution. By introducing several more logical design points, this method of expansion of a statistical design is known as design augmentation (69). For eg., by adding several further design points, a design following notable at two levels may be increased to a three-level design. It is possible to increase a design in a variety of ways, such as by replicating, applying core points to two-level structures, adding axial points (i.e. design points at multiple axes of the experimental region) or folding over.

2.5.3 Response surface

For a series of experiments conducted in a structured way to build a statistical model, one or more chosen experimental responses are reported during this critical stage in DoE (70). These methods include the postulation, with each response, of an analytical mathematical model that accurately reflects a shift in the response within the region of interest. Response surface modeling (RSM) includes integrating the coefficients into the model equation of a specific response variable rather than calculating the results of each variable directly, and projecting the response in the form of a surface over the entire experimental domain (71).

RSM is primarily a group of mathematical strategies for the development of analytical models and the exploitation of models. It attempts to connect a response to a variety of predictors influencing it by careful design and study of experiments by creating a response surface, which is an area of space. The independent variables representing the relationship of

these factors to the measured response are described within the upper and lower thresholds (72).

Response surface designs are experimental designs that allow the calculation of direct parameters, relative influence, and perhaps even quadratic effects and thus, give an understanding of the local form of response surface being investigated. In such conditions, it may be reasonable to characterize a response surface in a model that includes only significant interaction effects. These conditions emerge where no proof of "pure quadratic" curvature in the response of interest is revealed in the examination of the data - i.e. the response at the middle is nearly equal to the median response at the two severe stages, +1 and -1 (70).

The value of the response rises from the bottom of the figure to the top of each section of Figure 2-10 and those of the factor settings rise from left to right. If a reaction behaves as in Figure 2-10(a), only variables with two levels - low and high - ought to be used in the design matrix to measure the action. This paradigm is a fundamental precept in basic designs for two-level scanning or factor effect. The minimal number of levels needed for a factor to measure its action is three if an answer behaves as in Figure 2-10(b).

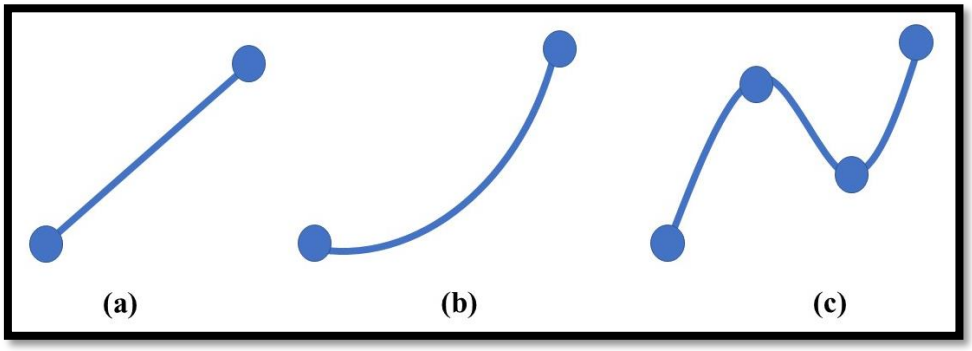


Figure 2-10 Different types of responses as functions of factor settings; (a) linear; (b) quadratic; (c) cubic

At this point, the addition of center points to a two-level architecture seems to be a sensible measure, but the layout of treatments in such a matrix can confuse all quadratic effects with one another. Only the quadratic aspect of the reaction can be detected by a two-level configuration with center points, but the actual pure quadratic results cannot be calculated. Quadratic structures for the development of drug delivery devices are commonly proposed (73). For DoE optimal protection, reaction surface designs containing experiments at three or more than three stages are also used. These reaction surface designs are used to define enhanced or optimal process conditions, address process issues and weak points, and render a more stable (i.e., less variable) formulation or process towards external and non-controllable influences. In

pharmacy research, comparatively more complex cubic responses (Figure 2-10(c)) are very infrequent.

2.5.4 Mathematical Models

An algebraic expression describing the dependency of an variable on the individual variable(s) is the mathematical model. Mathematical models can be conceptual or observational. An analytical model offers a way to identify the relationship of factor/response (74). Very commonly, but not necessarily, it is a set of polynomial function of a given order. The linear models most widely used are seen in following equations.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots. \quad \_(2)$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \ldots.- \quad 3)$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \ldots. + \varepsilon\_\_\_$$

Where y reflects the approximate answer, often referred to as E (y). The terms X indicate the value of the variables, and the constants describing the intercept, first-order/first-degree terms coefficients, second-order quadratic terms coefficients, and second-order interaction terms coefficients are β0, βi, βii and βij. Pure errors are indicated by the symbol. Equations (1) and (2) are linear variables, representing, in 3-D space, a smooth surface and a twisted plane. Equation (3) is a linear second-order construct representing a curved, twisted plane resulting from quadratic terminology. There can also exist or be suggested a theoretical framework or mechanistic framework. Very frequently, it is a nonlinear model where it is typically not easy to transform to a linear function (75). However, such theoretical relationships are seldom used in the production of pharmaceutical products.

2.6 PULMONARY DELIVERY OF PLHNCS

2.6.1 Dry Powder Inhalers:

Nebulizer, pressurized metered-dose inhaler (pMDI) and dry powder inhaler (DPI) are among the widely used systems for the pulmonary delivery of therapeutic agent. Among these, DPI emerges as most significant delivery systems for the pulmonary delivery due to its convenience and higher stability, more over DPIs are portable in nature, propellant-free and low-cost devices e.g. Spinhaler®. DPIs have to overcome various physical difficulties for effective drug delivery either local or systemic purposes (76). Different types of dry powder inhalers are shown in Figure 2-11.

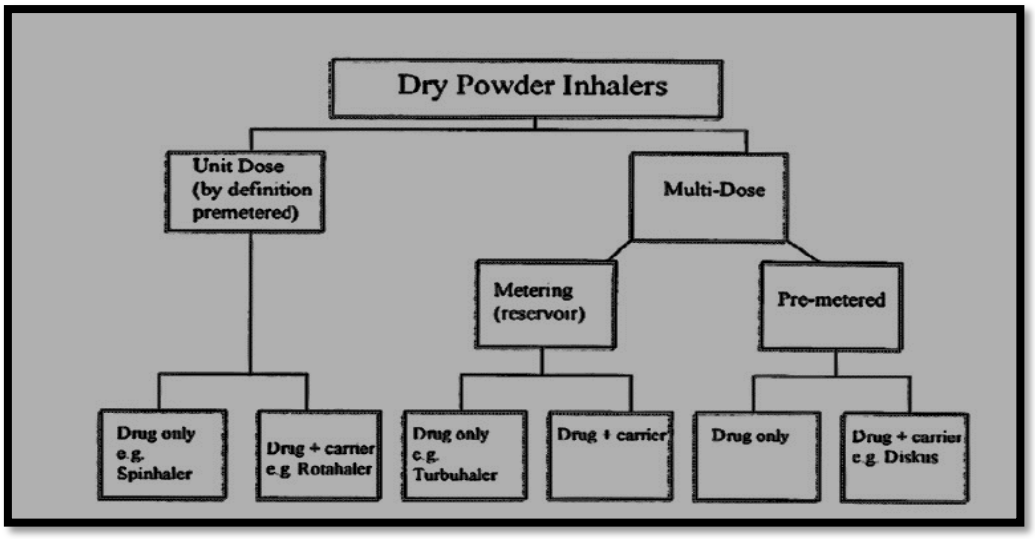


Figure 2-11 Types of Dry Powder Inhalers

2.6.2 Formulation of Dry Powder Inhalers:

Excipient used in the formulation of DPIs are based on the physicochemical properties of the drug and along with the target region of the DPI. Carriers, bulking agents, cryoprotectant and anti-adherents are used primarily for the formulation of the DPI.

2.6.2.1 The Role of Carrier in DPI Performance:

DPI has been formulated as a coarse powder mixture of particles with micronized drug particles having combined aerodynamic diameters of 1–5 μm. Carriers in the DPI are important to improve the dosage flowability to ensure the accurate dosing and minimum variability. Carriers are also effective in manufacturing and filling of the dosage form in to DPI capsules. Carriers particles are also effective for the emission of the dose from the device after punctuation (77). For the pulmonary delivery generally very small amount of drug is sufficient hence carriers are used to provide bulk with aim of enhancing handling, metering dose and dispensing. The presence of the carrier material is the taste/sensation on inhaling, which can assure the patient that a dose has been taken. Consequently, the carrier forms an important component of the formulation and any change in the physico-chemical properties of the carrier particles has the potential to alter the drug deposition profile (78). Therefore, the design of the carrier particle is important for the development of dry powder inhalations. Carrier particles should have several characteristics such as physico-chemical stability, biocompatibility and biodegradability. Further it should be compatible with the drug substance and must be inert, easily available and economical. During insufflation, the drug particles are detached from the surface of the carrier particles by the energy of the inspired air flow that overcomes the adhesion forces between drug and carrier. The larger carrier particles impact in the upper

airways, while the small drug particles go through the lower parts of lungs. Unsatisfactory detachment of drug from the carrier due to strong inter-particulate forces may be one of the main reasons of inefficient drug delivery encountered with most DPIs . Therefore, in the best case, the adjusted balance between adhesive and cohesive forces provides enough adhesion between drug and carrier to produce a stable formulation (homogeneous mixture with no powder segregation and proper content uniformity) yet allows for easy separation during inhalation. Consequently, it has been stated that the efficiency of a DPI formulation is extremely dependent on the carrier characteristics and the selection of carrier is a crucial for overall DPI performance.

2.6.2.2 Cryoprotectants/Bulking agents for DPI made by Lyophilization:

Bulking agents forms the bulk of the lyophilized product and provide an adequate structure to the cake. These are generally used for low dose (high potency) drugs that do not have the necessary bulk to support their own structure. These are particularly more important when the total solid content is less than 2 % (79). In such cases, a bulking agent is added to the formulation matrix. The freezing step of lyophilization is operated through cooling of shelves through the glass container in contact. The conduction, convection and radiation are the normal heat transfer modes during lyophilization. The sample has to be cooled from ambient conditions to sub-freezing temperature. During cooling first ice-nucleation occurs several degrees below equilibrium point of sample which is known as “supercooling”. Subsequent to nucleation, ice starts to grow and leads to freeze concentration of the sample consisting of two phase i.e. ice and freeze concentrated solution (80). The composition of such sample can be obtained by equilibrium freezing-curve of water in the presence of solute. In case of crystalline solutes, the solute crystallization occurs when temperature falls below the eutectic point.

The cryoprotectants play an important role in maintaining the characteristics of the lyophilized products. The aggregation of non-viral vectors is proposed to occur during the freezing step of lyophilization process, which can be avoided by using suitable concentration of Cryoprotectants (81). The particle isolation hypothesis based on separation of unfrozen particles in the unfrozen matrix has been proposed as probable mechanism of stabilization and there is no role of vitrification induced by polymers. After lyophilization the solid porous cake needs to be subjected to powderization process involving shear forces to convert them into free-flowing nature.

The powder processing of the lyophilized cakes needs to be performed to convert the poorly flowing mass of solid into a freely flowing, easy to fluidize powder bed which can be dispersed into air stream of respiratory tract in response to the actuation force by the patient

during inhalation (81). However, a systematic developmental approach involving through in vitro characterization is essential to convert them into practical applications. Different sugars has been described below as cryoprotectant:

2.6.2.2.1 Lactose:

Lactose, 4-(β-D-galactosido-)-D-glucose, can be obtained in either two basic isomeric forms, α- and ß-lactose, or as an amorphous form. Historically, lactose monohydrate was an obvious choice for use as a carrier excipient. Lactose accompanying with glucose and mannitol are used as carriers in DPIs by the US Food and Drug Administration department. Lactose is the most common and frequently used carrier in DPIs formulations and nowadays various inhalation grades of lactose with different physico-chemical properties are available in market. The advantages of lactose are its well-investigated toxicity profile, physical and chemical stability, compatibility with the drug substance, its broad availability and relatively low price. α-lactose monohydrate is the most common lactose grade used in the inhalation field (82).

2.6.2.2.2 Trehalose:

Trehalose dihydrate is a disaccharide sugar and crystalline hydrate like lactose. However, trehalose dihydrate is a non-reducing sugar and lactose monohydrate is a reducing sugar. As a reducing sugar, it participates in solid-state chemical degradation by the Millard reaction with certain types of small molecular weight drugs (such as formoterol and budesonide) and polypeptide/protein-type drugs. Hence trehalose as non-reducing sugar is more preferred over lactose as it doesn't take part in Millard reaction which is responsible for the degradation of sugars.

2.6.2.2.3 Sucrose:

Sucrose is having similar eutectic temperature i.e. -31°C (2%) as of lactose but it is not a reducing sugar and does not undergo Maillard reaction. Sucrose has a higher density as compared to lactose which can prevent collapse during drying (83).

2.6.2.2.4 Mannitol:

It is the most commonly and widely used excipients in the lyophilized products. Mannitol has a very high eutectic melting temperature (-1.5°C) after crystallization and lyophilization. Crystallization of the bulking agent, however, might adversely affect the physical stability of the product in certain instances, for which, an amorphous bulking agent is preferred.

2.6.3 Cascade Impactor-Inhaler Testing Equipment:

Cascade impactors operate on the base of inertial impaction. Each stage of the impactor contains a single or series of nozzles or jets through which the sample laden air is drawn directing any airborne particles settle towards the surface of the collection plate for that particular stage. Whether a particular particle impacts on that stage is dependent on its aerodynamic diameter. Particles having sufficient inertia will impact on that particular stage collection plate whilst smaller particles with insufficient inertia will remain entrained in the air stream and pass to the next stage where the process is repeated (83). The stages are normally assembled in a stack in order of decreasing particle size. As the jets get smaller, the air velocity increases and finer particles are collected. Remaining particles are collected on an after filter (or by a –Micro-Orifice Collector). The term 'Impactor' is generally used for an instrument where the particles 'impact' on a dry impaction plate or cup. If the collection surface is liquid, as in the case of the Multi-Stage Liquid Impinger (MSLI), then the term 'impinger' is used. The general principles of inertial impaction apply to both ‘impactors' and 'impingers'. The US and European Pharmacopoeia lists more than five different cascade impactors/impingers suitable for the aerodynamic assessment of fine particles. However, only the Andersen Cascade Impactor (ACI), the Next Generation Impactor (NGI) and the Multi-Stage Liquid Impinger (MSLI) appear in both pharmacopoeias. In research applications, in vitro/in vivo correlation and bioequivalence may be important and so detailed particle size data may be required. However, it is generally accepted that an impactor/impinger should have a minimum five stages and preferably more, if it is to provide detailed particle size distribution data. The aerodynamic particle size distribution of the drug leaving an inhaler device can define the manner in which an aerosol deposits in the respiratory tract during inhalation (84). This characteristic of the aerosol is often used in judging inhaler performance and is particularly relevant in the development of inhalation formulations during research, production, quality assurance and equivalency testing. The results of characterizations using cascade impaction techniques are additionally used for the determination of fine particle fraction or fine particle dose which may be correlated to the dose or fraction of the drug that penetrates to the lung during inhalation by a patient. Dry Powder Inhaler (DPI) testing could require added options for preventing stage overloading and necessary to achieve the specified pressure drop through the device. Upper stage mass overloading can be prevented with the addition of a high-capacity pre-separator or pre-collector. The feature traps non-inhalable aerosols. To achieve the proper pressure, drop of 4kPa (40.8 cm water) in the inhaler, a higher vacuum flow rate at 60 or 90 L/minute may be

needed. By analysing the drug deposited on the individual stages and the final filter, the Fine Particle Fraction (FPF), the Fine Particle Dose (FPD), the Mass Median Aerodynamic Diameter(MMAD) and Geometric Standard Deviation (GSD) can all be calculated (The Copley Scientific Limited, 2010) (85).

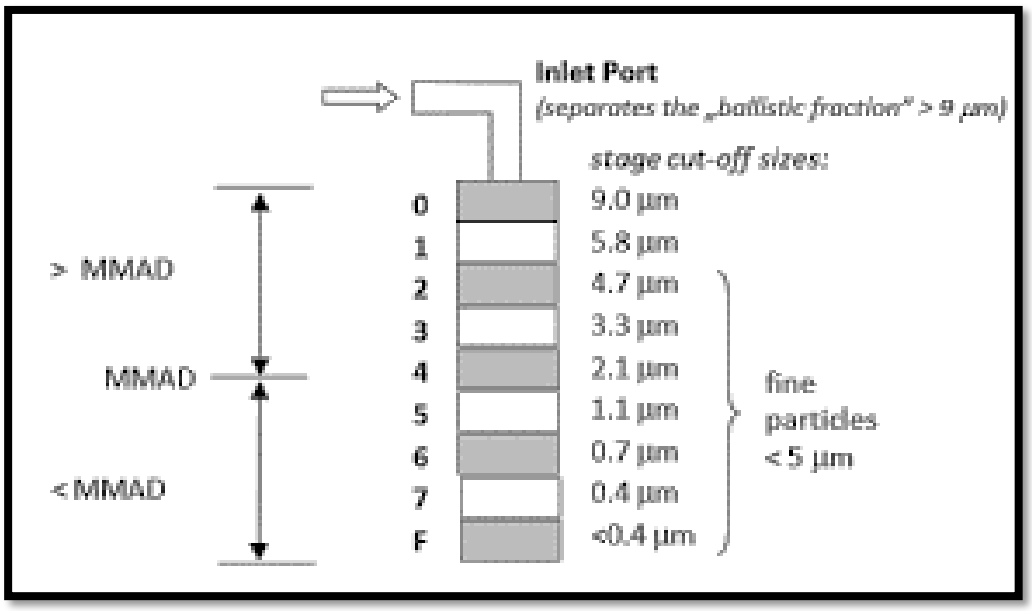


Figure 2-12 Particle size distribution and cascade impactor

2.6.4 Parameters used to define lung deposition:

The American and European pharmacopoeias have explained methods based on inertial impaction to assess the in vitro inhalation performance of formulations by determination of the fine particles.

2.6.4.1 Fine Particle Dose (FPD) And Fine Particle Fraction (FPF):

The aerodynamic evaluation methods of fine particles permit the determination of the fine- particle dose (FPD), which corresponds to the mass of drug particles that have an aerodynamic diameter less than 5 µm. Such particles can theoretically be deposited in the deep lung after inhalation. The fine-particle fraction (FPF), which is the percentage of the FPD usually related to either the nominal dose (total drug mass contained in the device) or the recovered drug (sum of the drug collected in the device and in the different parts of the impingers or impactors after inhalation) (86).

2.6.4.2 Emitted dose:

The emitted dose expresses the drug mass exiting the device after inhalation. In some cases, FPF can be calculated from emitted dose instead of total or recovered dose of drug from impingers or impactors. The ability of the powder to be fluidised by the airflow through an

inhaler is usually indicated by the emission dose, whilst the FPD and FPF measure the capability of the formulation to be fluidised and deagglomerated in time to release the drug from the carrier to be deposited in the appropriate level of the impactors and impingers.

2.6.4.3 Dispersibility:

The dispersibility is calculated as the ratio of FPD to emitted dose.

2.6.4.4 Mass median aerodynamic diameter (MMAD):

Mass median diameter of an aerosol means the particle diameter that has 50% of the aerosol mass residing above and 50% of its mass below it. The concept of aerodynamic diameter is central to any aerosol measurements and respiratory drug delivery. The aerodynamic diameter relates the particle to the diameter of a sphere of unit density that has the same settling velocity as the particle of interest regardless of its shape or density. The mass–mean aerodynamic diameter (MMAD) is read from the cumulative distribution curve at 50% point.

2.6.4.5 Geometric standard deviation (GSD):

The degree of dispersity is an important consideration for both quality and efficacy of pharmaceutical aerosols. The nature of the aerosol distribution must be established accurately if its implications for deposition and efficacy are to be understood. The degree of dispersion in a log normally distributed aerosol is characterized by the geometric standard deviation (GSD). A larger GSD implies a large particle size tail in the distribution. GSD for a well-functioning stage should ideally be less than 1.2 (the GSD for an ideal size fractionator would be 1.0 and indicates a monodisperse aerosol. GSD is a measure of the variability of the particle diameters within the aerosol and is calculated from the ratio of the particle diameter at the 84.1% point on the cumulative distribution curve to the MMAD. For a log-normal distribution, the GSD is the same for the number, surface area or mass distributions (87).

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