Chapter – 7

Preparation and optimization of Dry Powder Inhalers (DPIs) of HA grafted liposomes

Chapter 7 Preparation and Optimization of Dry Powder Inhalers (DPIs) of HA grafted liposomes

INTRODUCTION

Aerosolized administration of drugs to the lung has generated an appreciable deal of interest for many years to treat the localized bronchial diseases like asthma. Inhalational route can serve as an excellent route for localized and targeted delivery as it reduces non specific drug distribution and enhances drug distribution in site of interest. Hence, this route of delivery cuts the edge over oral and other conventional routes of administration. Bronchodilators, anti-inflammatory agents, mucolytics, antiviral agents and anticancer agents have been widely studied and even commercialized as aerosolized formulations.

Aerosolization of novel drug delivery systems such as nanoparticles and liposomes can further enhance stability and performance profile of bioactives against adverse microenvironmental factors existing at the site of action. However, factors like particle size, density, flowability, deposition and retention of drug in lung etc. are major constraints that mar the popularity of inhalational route of administration. Particle size is a critical factor to determine the performance of the inhalational dosage forms in terms of delivery efficiency and rate. It is generally accepted that aerosol particles of 1–5 μm size are required for deposition in the alveolar region of the lung, the region of the highest absorption. Commonly observed problems such as particle aggregation may adversely affect the properties of inhalational dosage forms. Though aerosols comprise one of the most desirable and popular means of drug administration to lungs, drug targeting by aerosolization is still a long way to go. Inhalational drug therapy mainly comprises of three systems for drug administration viz. pMDI (pressurized metered dose inhalers), Nebulizers and DPIs (Dry Powder Inhalers). Each of these means of administration has got their own set of merits and demerits.

1. pMDI: the drug is either suspended or dissolved in a propellant and filled under pressure into a canister. Release of a predetermined volume of fluid causes expansion of a propellant followed by evaporation resulting in dry aerosol particles suitable for inhalation.
2. Nebulizers: They are usually powered by compressed air or high frequency sound waves to generate aerosolized form.

3. DPIs: Produce small sized particles. DPIs are usually activated by shearing action applied by patients during inhalation. Application of shearing stress caused segregation of particles that usually remain adhered to cryoprotectant/carrier.

An ideal inhalation system has to produce a particle-size distribution suitable for efficacious delivery to the lungs. Ideally, the diameter of the aerosol particles or droplets should be in the range of 1 μm to 5 μm (respirable fraction) since particles in size range of 1-5 μm can be easily deposited in interiors of lungs by sedimentary forces.

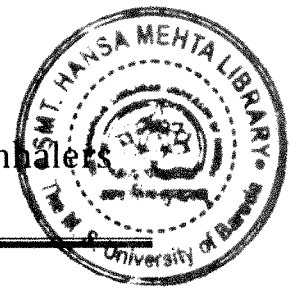
- One of the major limitations of existing inhaled drugs is mainly the transient drug action resulting in shorter duration of action leading to frequent drug administration ending up in poor patient compliance and high incidence of dose dependent adverse drug reactions. Various strategies have been proposed and worked out by different groups to overcome the limitations of these short acting drug candidates.

Drug formulation plays an important role in producing an effective inhalable medication. A drug designed to treat a systemic disease must be deposited and retained in the lungs to ensure optimum bio availability. Thus, a formulation that can be retained in the lungs for the desired period of time, while avoiding the clearance mechanisms of the lung, can prove to be an ideal one. Few of such approaches are: encapsulation of drugs in bio degradable polymer based microspheres, nanoparticles, liposomes or chemical modification producing prodrugs with longer residence time, drug complexes with cyclodextrins etc. Liposomes have been studied for years as a pulmonary drug delivery vehicle and used as a means of delivering bio actives to the alveolar surface for treatment of respiratory disorders. Versatility, bilayer fluidity, appreciable encapsulation efficiency etc. render liposomes ideal candidates for preparation of DPIs. Liposomes have been thoroughly investigated and explored for their potential in achieving controlled and site specific drug delivery. Liposomal aerosols offer a wide spectrum of advantages in lung targeting in terms of suitability and computability for lipophilic drugs, enhanced drug potency and site specificity, prevention of local irritation, uniform drug deposition, reduced dose frequency and hence, improvement in overall patient compliance. Liposomes can be successfully used for encapsulation of hydrophilic as well as lipophilic drugs

and have been used for encapsulation of cytotoxic agents, anti asthmatic drugs, chemotherapeutic agents for treatment of infections etc. (Zeng, 1995)

Though liposome aerosols have attracted a good deal of attention from researchers in this area, especially in the field of nebulizers (Taylor et al, 1989 and 1990) and dry powder inhalers, (Schreier et al, 1994 and Ho, 1995) stability and drug leakage issues constrain the popularity of aerosol based formulations. (Niven, 1992) Use of chlorofluorocarbon based propellants in liposomal aerosols has also raised serious environmental concerns and hence, liposomal DPIs can be potential substitutes for aerosol based formulations (Schreier et al, 1994, Ho, 1995 and Holzner et al, 1998) DPIs are drug delivery devices that contain solid drug, suspended or dissolved in a dry powder mix (DPI) that is fluidized when the patient inhales. Barry and O'Collahgan and Dolovich et al have reviewed the studies of performance of inhalational devices used in clinical trials and found them to be clinically superior in terms of patient friendliness, eco friendliness, patient compliance and feasibility. Higher velocity of metered dose inhalers results in premature drug deposition in oropharynx region and lack effective lung delivery. (Newman, 1993 and Ganderton, 1997) and thus requires careful co ordination of actuation and inhalation. Since, DPIs are activated by patient's inhalational airflow, they do not require any actuation or co ordination resulting in substantially greater drug deposition in lungs.(Borgstorm, 1996). DPIs also offer higher stability as they are usually formulated as single phase, solid particulate blends. (Ashurst, 2000) and their lower energy state reduces the rate of degradation in contrast to metered dose inhalers and aerosols that employ the use of different types of organic solvents. Powder properties such as morphology, surface texture, distribution of particle sizes and aerodynamic behaviour largely dictate the performance of DPIs and need to be carefully addressed.

Micronization of liposomes in size range of 1-5 μm along with lyophilization yields a stable dosage form that can effectively target lungs and can penetrated to deeper peripheral areas and interiors of lungs, particularly alveoli and deeper regions of bronchi (Mobley et al, 1994 and Sun et al, 1996). Freeze drying or lyophilisation also yields a stable product that can be further



processed down to DPI. However, freeze drying is considered to be stressful for liposomes or any nanoconstruct and can result into structural distortion of liposomes. Madden and Co-Workers, 1985 for the first time addressed the stability issue pertaining to liposomes and found that liposomes can be reduced to dry powders if they are freeze dried in the presence of certain sugars. Hence, various cryoprotectants have been studied to prevent structural distortion of liposomes. Carbohydrates have been found to play excellent role in stabilizing the liposomes during freeze drying. It has been proposed that sugars can align in between the phospholipid head of lipid molecules of liposomes and can replace the bound water by forming hydrogen bonds thereby maintaining the structural integrity of liposomes. (Crowe et al, 1973). On addition of carbohydrates to liposomal dispersions, they form a glassy matrix during freezing and prevent fusion of the vesicles and provide protection against ice formation (Edward et al, 1997). Redispersibility, particle size, tendency to aggregate, surface texture of freeze dried liposomes (now DPIs) are critical factors that determine DPI performance. (French, 1996) Studies have shown that particles with low bulk density, large volume mean diameter can be more efficacious in drug delivery to lungs. (Chougule, 2007, Edwards et al, 1997 and Edwards et al, 1998).

The efficacy of DPI in effective drug delivery to lungs can be improved further by smoothening the carrier surface (Ganderton et al, 1992), reducing the particle size of the carrier (French et al, 1995 and Steckel et al, 1997) and use of ternary powder mix formulation (Staniforth et al, 1996).

In cancer chemotherapy, cytotoxic drugs are administered by parenteral routes to kill cancerous cells. However, parenteral administration of anti cancer agents unleashes series of severe adverse drug reactions owing to their non specific drug distribution. This non specificity of anti cancer drugs and their failure to differentiate among normal healthy host cells and cancer cells results in plethora of adverse reactions such as skin, gastrointestinal, and bone marrow ailments. Inhalational administration of anti cancer drugs provide a means to render the drug more site specific and preferentially avoid non specific drug distribution. Among various strategies employed for targeted delivery of cytotoxic agents to lung cancer, direct tumour targeted aerosolized delivery of chemotherapeutic agent, alone or in combination with other drugs seems

to be an innovative and potential approach for the treatment of lung cancer. Recently, nebulized liposomal formulations of 9-nitrocamptothecin (9-NC) and paclitaxel have been studied in the treatment of lung cancer in animal models. Knight et al. tested the anticancer properties of liposomes containing camphothecin and its analogue (9-NC) in different animal models. However, need for a feasible and viable alternative to ozone depleting and non eco friendly metered dose inhalers, coupled with the opportunity for dehydrating liposomes to powder form, render dry powder inhaler (DPI) of liposomal drug an attractive choice for modulated inhalation drug delivery.

In the current study, we hypothesized to develop aerodynamically light and porous liposomal Dry Powder Inhaler formulations (Liposomal DPIs) using freeze drying techniques with minimal drug leakage, improved pulmonary deposition, and prolonged drug residence at site of action. The developed liposomal DPIs were characterized for particle size, in vitro lung deposition, moisture content, flow properties so as to develop a formulation that can effectively target lung cancer.

Chapter 7 Preparation and Optimization of Dry Powder Inhalers (DPIs) of HA grafted liposomes

7.1 MATERIALS AND METHODS

Materials	Source
Glassware: 10 ml pipette, serological pipettes, beakers, conical flask, glass vials, funnel,spatula, burette, burette clamp	Durga Scientific Traders, Vadodara,India
Cryovials	Tarsons Ltd., Kolkatta, India.
Lactose, sucrose, mannitol	Croda Healthcare, U.K.
L-Glycine	Free Gift sample from Alembic Ltd., Vadodara, Gujarat, India.
Dialysis bag (Mol wt. cut off: 1000)	Sigma Aldrich Corporation, Mumbai, India.
Equipments	Source
Lyophilizer, DW1, 0-60E	Heto Drywinner, Denmark
Bulk Density Apparatus	Labindia, Mumbai, India.
UV Visible Spectrophotometer	Shimadzu Uv-1601, Japan.
Andersen cascade impactor with a preseparator	Graseby-Andersen, Atlanta, GA, USA
2" Hard Gelatin Capsules	Universal Capsules, Mumbai, India.
Inhalator (Rotainhaler)	Cipla Ltd., Mumbai, India.
Scanning Electron Microscope	Jeol, Japan
Particle size Analyzer	Malvern Instruments, U.K.

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7.2 FREEZE DRYING OR LYOPHILIZATION OF HA GRAFTED ETOPOSIDE LOADED LIPOSOMES

Freeze drying technique was used for conversion of optimized liposomes to DPIs followed by their stabilization and to achieve desired properties for pulmonary administration. Etoposide loaded liposomes (ETPLIP) were prepared by Lipid Film Hydration or Thin Film Hydration technique (Chapter-4) using HSPC: DPPE: CHOL-8:1:1 followed by grafting with Hyaluronic acid (HA) by carbodiimide coupling technique (Ch 5) and freeze dried with different cryoprotectants and 15% w/w glycine as anti adherent to preserve the vesicular size and shape, hence the PDE. The liposomes of optimized batch of above composition were selected for development of freeze dried Liposomal DPI.

In HA grafted liposomal suspension of Etoposide batch equivalent to 4 mg, 400 mg of different carriers and 15 % L-glycine of total mass (500 mg) were dissolved. The resultant dispersion was frozen overnight at -40°C, dried for 48h at 50mbar using a Heto Freeze-dry system (Hetodry, Denmark).The porous cake thus formed was sized successively through #120 and #240 sieves. PDR of freeze dried liposomes were determined following dehydration-rehydration cycle. Details related to Etoposide liposomal DPI composition are given in Table 7.1. Influence of cryoprotectants on particle size and percentage drug retention was studied and results are shown in Table 7.2.

Table 7.1: Compositions and abbreviations for different lyophilized liposomal formulations of ETP

Sr. No.	Name and code of formulation	Carrier* added
1.	Lyophilized Etoposide plain drug with lactose (LEPL)	Lactose
2.	Lyophilized Etoposide Liposomal Dry Powder Inhaler with lactose (LEDPIL)	Lactose
3.	Lyophilized Etoposide Liposomal Dry Powder Inhaler with sucrose(LEDPIS)	Sucrose
4.	Lyophilized Etoposide Liposomal Dry Powder Inhaler with Mannitol(LEDPIM)	Mannitol

*-Glycine was added in proportion of 15% w/w of total formula weight of liposomal dispersion.

Table 7.2 Influence of different cryoprotectants on freeze dried Liposomal DPI

Formulation codes	Cryoprotectant (shown in bold) studied	VMD (μ)*	Drug retention (%)*
LEPL	Plain Etoposide with lactose + 15 % w/w glycine	10.1 ± 2.10	95.35 ±3.35
LEDPIL	Etoposide liposomes+**lactose**+15 % w/w glycine	9.1 ±1.76	97.12 ± 2.1
LEDPIS	Etoposide liposomes + **Sucrose**+ 15 % w/w glycine	11.43 ±1.80	97.35 ± 0.97
LEDPIM	Etoposide liposomes + **Mannitol**+15 % w/w glycine	5.9 ±1.01	97.23 ± 0.86

*-**Mean ± S.D. (n=3)**

7.3 FREEZE DRYING OR LYOPHILIZATION OF HA GRAFTED DOCETAXEL LOADED LIPOSOMES

Freeze drying technique was used for conversion of optimized liposomes to DPIs followed by their stabilization and to achieve desired properties for pulmonary administration. Docetaxel loaded liposomes (DOCLIP) were prepared by Lipid Film Hydration or Thin Film Hydration technique (Chapter-4: section 4.2.) using HSPC: DPPE: CHOL-9:2:1 followed by grafting with Hyaluronic acid (HA) by carbodiimide coupling technique (Ch 5) and freeze dried with different cryoprotectants and 15% w/w glycine as anti adherent to preserve the vesicular size and shape, hence the PDE. The HA grafted liposomes of optimized batch of above composition were selected for development of freeze dried Liposomal DPI.

In HA grafted liposomal suspension of Docetaxel batch equivalent to 3.5 mg, 450 mg of different carriers and 15 % L-glycine of total mass (500 mg) were dissolved. The resultant dispersion was frozen overnight at -40°C, dried for 48h at 50mbar using a Heto Freeze-dry system (Hetodry, Denmark). Details related to Etoposide liposomal DPI composition are given in Table 7.3. The effect of use of different cryoprotectants and anti adherent on particle size and PDR of developed Liposomal DPI of Docetaxel was studied and reported in Table 7.4. The porous cake thus formed was sized successively through #120 and #240 sieves. PDR of freeze dried liposomes were determined following dehydration-rehydration cycle.

Table 7.3: Compositions and abbreviations for different lyophilized liposomal formulations of DOC

Sr. No.	Name and code of formulation	Carrier added*
1.	Lyophilized Docetaxel plain drug with lactose (LDPL)	Lactose
2.	Lyophilized Docetaxel Liposomal Dry Powder Inhaler with lactose (LDDPIL)	Lactose
3.	Lyophilized Docetaxel Liposomal Dry Powder Inhaler with sucrose(LDDPIS)	Sucrose
4.	Lyophilized Docetaxel Liposomal Dry Powder Inhaler with Mannitol(LDDPIM)	Mannitol

*-Glycine was added in proportion of 15% w/w of total formula weight of liposomal dispersion.

Table 7.4 Influence of different cryoprotectants on freeze dried Liposomal DPI

Formulation codes	Cryoprotectant (shown in bold) studied	VMD (μ)*	Drug retention (%)*
LDPL	Plain Docetaxel with lactose + 15 % w/w glycine	11.3 ± 2.5	90.12 ± 4.86
LDDPIL	Docetaxel liposomes+**lactose**+15 % w/w glycine	9.9 ±0.8	93.41 ± 1.36
LDDPIS	Docetaxel liposomes + **Sucrose**+ 15 % w/w glycine	10.5 ±1.80	97.12 ± 1.15
LDDPIM	Docetaxel liposomes + **Mannitol**+15 % w/w glycine	5.5 ±1.2	99.40 ± 0.74

*-Mean ± S.D. (n=3)

7.4 CHARACTERIZATION OF LIPOSOMAL DPI:

The Liposomal DPIs were characterized for the following physico-chemical properties.

7.4.1 Angle of repose

The pile of powder was carefully built up by dropping the powder material through a funnel tip till from height of 2cm (Carr, 1965). The angle of repose was calculated by inverting tangentially the ratio of height and radius of the formed pile (Table 7.5 and 7.6)

7.4.2 Compressibility index

Compressibility index (CI) of powder as a measure of flow and dispersibility were measured by methods as described in United States Pharmacopoeia, (2001)

The compressibility index was calculated by the following formulae:

$$\frac{100\,(V_0 - V_f)}{V_0} \qquad \text{----------(1)}$$

Where V_0 is the initial volume of the weighed sample &

V_f is the final volume of the sample after 500 taps.

The CI for Liposomal DPIs are summarized in Table 7.5 and 7.6.

7.4.3 Tapped Density

Tapped density was determined by mechanically tapping a measuring cylinder containing 1 g of powder sample. After observing the initial volume, the cylinder was mechanically tapped, and volume reading was taken until little to no change in volume was observed. The plateau condition was obtained after 500 taps for all samples. The tapped densities of Liposomal DPIs are summarized in Table 7.5 and 7.6.

7.4.4 Residual water content

The residual water content of prepared HA grafted liposomal DPI (1g) was determined by Karl-Fischer titration. Commercially available pyridine free reagent was used analysis. The reagent was standardized with addition and determination of known quantity of water (250mg). Initially, 40ml of methanol was added into the titration vessel and titrated with the reagent to determine the amount of water present in the samples. The water content determined for the Liposomal DPI is recorded in Table 7.5 and 7.6.

Table 7.5 Solid State Characterization and Residual Water Content of Lyophilized Formulations (HA grafted liposomal Etoposide DPI)

Formulation code	Tapped density* (g/cc)	Angle of repose (^{0})*	Carr's Compressibility index*	Residual water content (%)*
LEPL	0.81± 0.2	45.6±3.1	21.1± 1.9	6.8 ±1.1
LEDPIL	0.24±0.05	33.8±2.7	36.2±2.3	5.1±0.85
LEDPIS	0.38±0.14	35.1±1.9	30.9±3.1	5.4± 0.86
LEDPIM	0.16 ± 0.02	25 ±1.16	38.5±1.98	2.46±0.33

***Mean±S.D. (n=3)**

Table 7.6 Solid State Characterization and Residual Water Content of Lyophilized Formulations (HA grafted liposomal Docetaxel DPI)

Formulation code	Tapped density* (g/cc)	Angle of repose (^{0})*	Carr's Compressibility index*	Residual water content*
LDPL	0.81± 0.2	45.6±3.1	20.8± 2.2	7.3 ± 0.98
LDDPIL	0.31±0.41	34.7±1.4	35.2±1.5	5.4 ±0.62
LDDPIS	0.34±0.11	35.4±2.3	31.6±1.4	5.2± 0.77
LDDPIM	0.20 ± 0.04	26.1 ±0.97	39.1±1.65	2.46±0.84

***Mean ± S.D. (n=3)**

7.4.5 Particle size determination

Particle sizes of rehydrated liposomes particle sizes and the Mass median Aerodynamic diameter of liposomal DPI formulations were determined as discussed below.

The particle size of Liposomal DPI was determined by laser diffraction using Malvern particle size analyzer. The liposomal DPI formulations were dispersed in isopropyl alcohol and stirred at 2000 rpm in order to reduce the interparticle aggregation, and laser obscuration range was maintained between 10-20%. The mean mass aerodynamic diameter (MMAD) was determined by the following formula and reported in Table 7.8 and 7.9.

d_{aer} (MMAD) = $\rho^{-1/2}$ x d (VMD) ----------(2)

where, ρ is tapped density in units of g/cm^{3}and d is volume mean diameter in micron

7.5 CHARACTERIZATION OF AEROSOL PERFORMANCE

The *in- vitro* pulmonary deposition was evaluated using an eight stage, nonviable Andersen cascade impactor with a preseparator (Graseby-Andersen, Atlanta, GA, USA) operating at an airflow rate of 28.3 l/min. A size '2' hard gelatin capsule was filled with 35 mg of powder equivalent to 500 µg of ETP and aerosolized using Inhalator. Ten capsules were actuated for each impaction with each capsule for 10 secs. The drug content deposited in different parts such as induction port, preseparator, individual impaction plates, and powder remaining in capsule and inhaler device was rinsed with methanol. From drug deposition data the emitted dose, fine particle dose, fine particle fraction, mass median aerodynamic diameter, and geometric standard deviation were calculated according to USP 27 NF 22.

Fine particle dose was calculated from ratio of the total mass of powder (R) having particle size below 5 µm, found on the stages of Cascade Impactor apparatus and filter to the number of dose (n).

$$Fine\ particle\ dose = R/n \quad \text{---------- (3)}$$

Fine particle fraction was calculated from the ratio of R to the total mass (ΣA) of powder delivered from the mouthpiece of the inhaler into the apparatus i.e

$$Fine\ particle\ fraction = R/\Sigma A \qquad \text{---------- (4)}$$

- Recoverable Dose is the total quantity of drug recovered per capsules after each actuation.
- Emitted dose is the drug emitted from the inhaler device.
- % Emission is the ratio of emitted dose to total dose.
- % Dispersibility is the % of FPD to emitted dose.

The results of aersolization performance of developed Liposomal DPI are summarized in Table 7.7 and 7.8.

Table 7.7 Summary report of in vitro drug deposition of HA grafted liposomal Etoposide DPIs prepared by lyophilization

Formulation code	Emitted Dose (%)*	%FPF(Fine Particle Fraction)*	Mean Mass Aerodynamic Diameter (MMAD)* (μ)	% Dispersibility*
LEDPIL	72.6 ± 2.2	67.23 ± 1.57	2.71 ±0.31	47.56 ± 3.68
LEDPIS	67 ± 0.18	50.76 ± 3.16	2.41 ±0.63	59.78 ± 6.13
LEDPIM	85.12± 0.77	76.75 ±2.16	2.1 ±0.31	61.24 ± 5.44

***Mean±S.D. (n=3)**

A size '2' hard gelatin capsule was filled with 35 mg of powder equivalent to 250 μg of DOC and aerosolized using Rotahaler. Ten capsules were actuated for each impaction with each capsule for 10 secs. Results were recorded in Table 7.8.

Table 7.8 Summary report of in vitro drug deposition of HA grafted liposomal Docetaxel DPIs prepared by lyophilization

Formulation code	Emitted Dose (%)*	Fine Particle Fraction (FPF %)	Mean Mass Aerodynamic Diameter (MMAD)* (μ)	% Dispersibility*
LDDPIL	65.58 ± 0.16	36 ±1.37	2.88 ± 0.72	44.5 ± 2.71
LDDPIS	72.01 ± 0.27	54.39 ± 1.22	2.57 ± 0.36	55.23 ± 1.45
LDDPIM	78.08 ± 0.16	70.41 ± 1.46	1.99 ± 0.24	59.78 ± 2.16

***Mean±S.D. (n=3)**

7.6 SURFACE MORPHOLOGY ASSESSMENT OF DPIs

The surface morphology of liposomal DPIs of Etoposide and Docetaxel was examined by scanning electron microscopy (SEM) (JSM-5610 LV, JEOL, Japan). Powder samples were adhered to sample stubs using double sided adhesive tape, and then viewed using an accelerating voltage of 15 kV at the magnification of ×250 to ×5,000 and shown in Fig 7.1 and Fig 7.2.

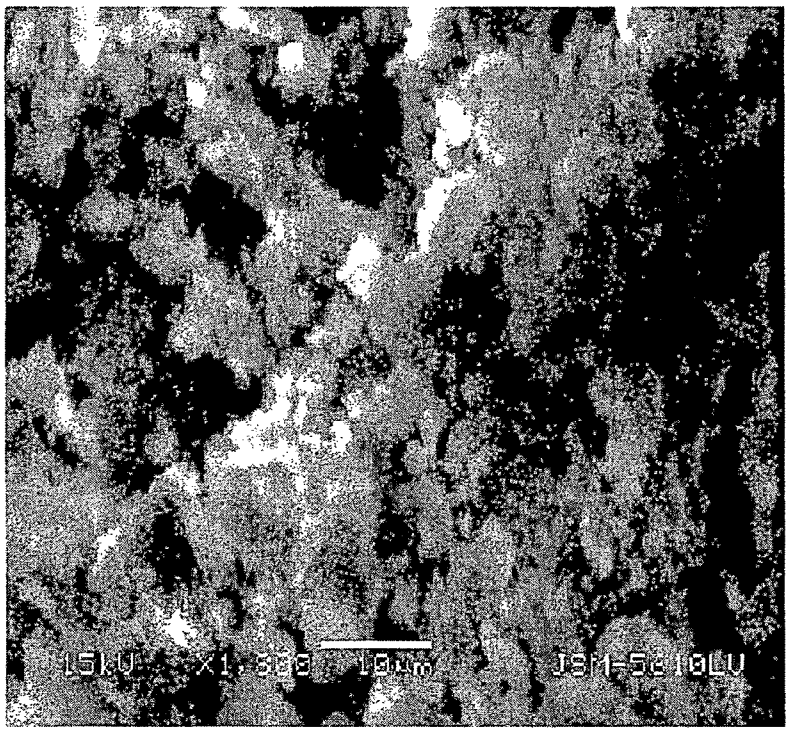

Fig 7.1 : SEM image of Liposomal Etoposide DPI

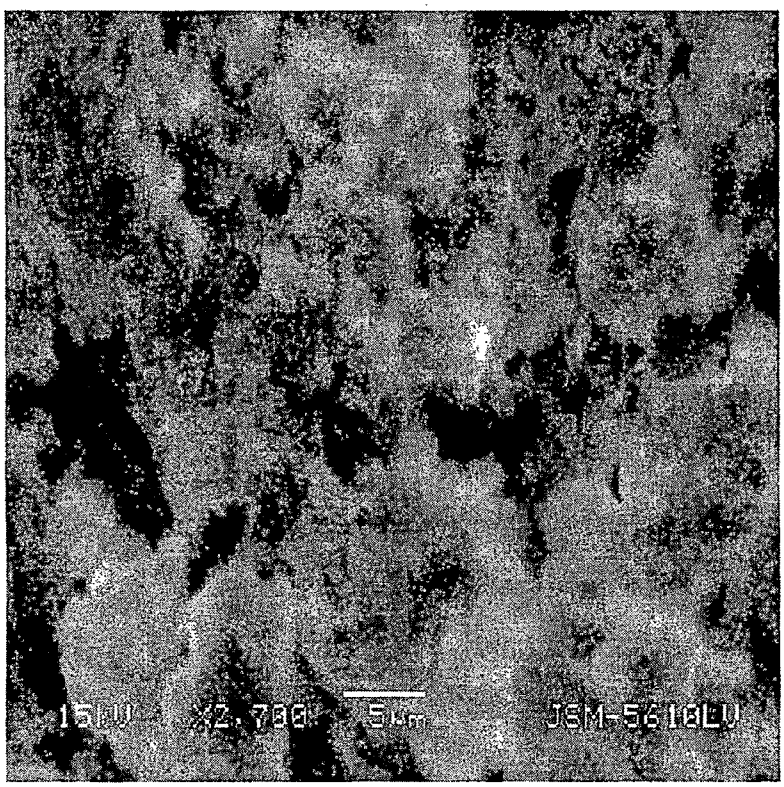

Fig 7.2 : SEM image of Liposomal Docetaxel DPI

7.7 *IN VITRO* RELEASE STUDIES

In vitro release studies of developed DPI formulations and lyophilized plain ETP and DOC with lactose were evaluated using a diffusion cell across cellophane membrane (10,000 MCO) for 48 h using 50 ml of mixture of phosphate buffer saline (PBS) and methanol (6:3) as diffusion medium at 37°C. The formulations equivalent to 15 doses; 300 μg ×15 of ETP were dispersed in methanolic PBS. Formulations to be compared were separately transferred to the donor compartment and stirred at 50 rpm while the receptor compartment was stirred at 100 rpm. 1ml of the aliquot was withdrawn from the receptor compartment at definite time intervals and replaced with equal volume of fresh medium. Drug content in aliquots was evaluated

spectrophotometrically Results of in vitro drug release for liposomal DPI of Etoposide are shown in Fig 7.3 and Table 7.9 while those of Docetaxel are shown in Fig 7.4 and Table 7.10.

Table 7.9: In vitro drug release profile of plain Etoposide and developed HA grafted liposomal DPIs of Etoposide with different carriers.(Results expressed as Mean ±S.D., n=3)

Time in hrs.	LEPL	LEDPIL	LEDPIS	LEDPIM
0.5	20.56 ± 0.76	4.4 ± 0.09	2.1± 0.87	0.96±0.11
1	46.12 ± 1.05	7.3±0.96	3.6± 0.34	1.93± 0.13
1.5	70.14± 0.95	9.2±0.53	5.2±1.74	3.4±1.12
2	81.65± 2.13	13.5±2.43	8.16± 0.99	7.01±1.68
3	97.44± 3.44	17.67±2.67	14.78±1.98	11.89± 2.13
4		24.54±3.12	23.18±2.65	16.18±2.12
6		32.51±2.51	38.97±3.24	23.91± 1.76
8		41.69±1.68	50.05±4.37	31.24±1.88
10		57.13±0.97	62.14±1.87	43.18± 2.13
12		72.48±1.24	71.18±2.11	53.79± 3.14
18		84.17±1.95	85.33±3.09I	65.43± 1.27
24		89.23±2.31	92.18±0.12	73.31± 0.98
36		94.56±3.12	97.65±2.77	87.52±1.61
48		98.24±1.73		97.3± 1.73

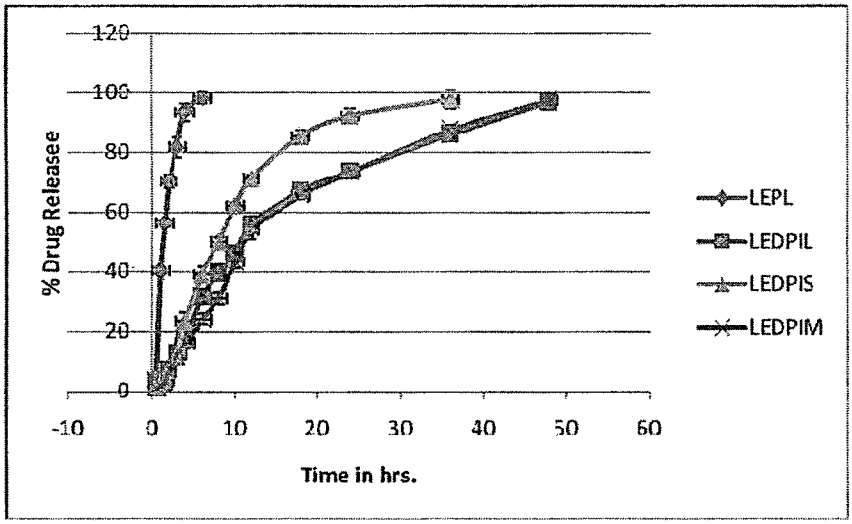

Fig 7.3 In vitro drug release profile of plain Etoposide and developed HA grafted liposomal DPIs of Etoposide with different carriers.(Values expressed as Mean ± S.D.,n=3)

Table 7.10: In vitro drug release profile of plain Docetaxel and developed HA grafted Docetaxel liposomal DPIs with different carriers.

Time in hrs.	DEPL	LDDPIL	LDDPIS	LDDPIM
0.5	24.85 ± 3.88	5.1 ± 0.97	3.0± 0.43	1.13±0.21
1	42.33 ± 2.91	8.2±1.05	5.5± 0.27	2.24± 0.61
1.5	68.68±1.65	14.2±1.06	6.7± 0.71	3.97±1.09
2	87.58± 2.08	21.57±1.43	9.11± 0.42	5.98± 0.76
3	97.14± 2.56	29.37±1.23	15.33± 2.01	10.76± 0.98
4		37.54± 2.76	20.28±1.81	15.33± 1.65
6		45.65±1.18	32.67± 2.58	24.68± 1.86
8		52.69±1.68	43.88 ± 3.08	33.29±1.61
10		61.13±0.97	52.94±1.34	45.26± 1.90
12		73.40±1.89	63.23±1.96	54.82± 1.45
18		85.22±1.57	80.46 1.99	67.21± 1.23
24		93.57± 3.11	90.61± 1.39	75.81± 0.75
36		98.89 ±1.98	96.98± 3.44	88.44±2.59
48				98.84± 2.98

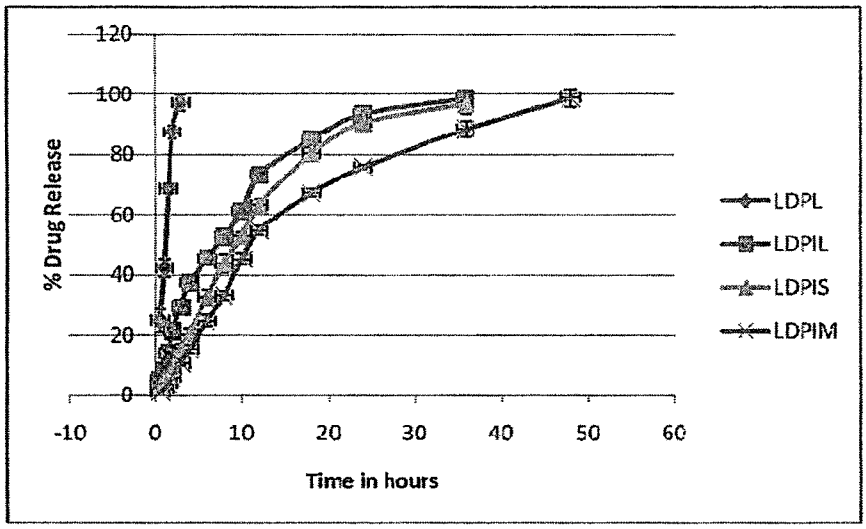

Fig 7.4- In vitro drug release profile of plain Docetaxel and developed HA grafted Docetaxel liposomal DPIs with different carriers.(Values are expressed as Mean ±S.D., n=3)

7.8 DISCUSSION

Lyophilization and spray drying techniques have been widely adopted for preparation of liposomal DPIs. However, freeze drying or lyophilization technique offers salient advantages over the spray drying technique in terms of stability, batch size etc. (Mobley, 1994 and Sun,1996) and hence, was preferred to convert HA grafted drug (s) loaded liposomes to DPIs. Nanometric size range of liposome facilitates uniform distribution in bulk of dry powder formulations. Lyophilization process parameters play a vital role on properties of DPIs such as particle size, shape, topographical features, density, moisture content, and drug retention. Hence, process parameters were optimized for development of DPIs and operating parameters were kept constant during preparation of all DPIs. The lyophilized powder yield was observed in between 65% and 72%.

Lyophilized ETPLIP with lactose (LEDPIL), lyophilized ETPLIP with sucrose (LEDPIS) and lyophilized liposomes with mannitol (LEDPIM) were found to have VMD (Volume Mean

Diameter) of 9.1±1.76, 11.43±1.80, and 5.9±1.01 μm respectively, whereas LEPL was found to have VMD of 10.1±2.1 μm, while lyophilized DOCLIP with lactose (LDDPIL), lyophilized DOCLIP with sucrose (LDDPIS) and lyophilized DOCLIP with mannitol (LDDPIM) were found to have VMD of 9.9 ± 0.8 μ, 10.5 ± 1.80 and 5.5 ± 1.2 μ respectively while that of lyophilized plain DOC with lactose (LDPL) was found to be 11.3 ±2.5 μ indicating that minimum VMD was observed in case of Docetaxel DPIs prepared using mannitol which can ensure good penetration and retention in lungs as size of powder is around 5 μ in case of LDDPIM as compared to DPIs formulated using sucrose and lactose.

All the Dry Powder Inhaler formulations (DPIs) exhibited more than 95% drug retention on an average. Along with the afore discussed parameters, innovative means and techniques of developing aerodynamically light particles have been engineered and studied to facilitate drug optimum drug deposition in deeper parts of lungs.(Tsai, 2004). It has been observed that DPI formulations with tap density lesser than 0.4 g/cc and relatively large mean diameter falling in range of 5-30 μm, however, possess MMAD (Mean Mass Aerodynamic diameter) of 1-5 μm. The LEDPIM was found to have the lowest density (0.16±0.02 g/cc), good flowability (Angle of repose 25.0±1.16°, Carr's compressibility index 38.5±1.98 %), and low residual water content of 2.46±0.33. The LEDPIL was found to have density of 0.24±0.05 g/cc, angle of repose of 33.8±2.7°, Carr's compressibility index of 36.2± 2.3%, and residual water content of 5.1±0.85 and LEDPIS with tapped density of 0.38±0.14 g/cc, angle of repose of 35.1±1.9°, Carr's compressibility index of 30.9±3.1%, and residual water content of 5.44±0.86. Similarly, LDDPIM was found to have the lowest tap density of 0.20 ±0.04 g/cc, angle of repose of 26.1 ± 0.97 ^{0} Carr's compressibility index of 39.1 ± 1.65 and residual water content of 2.46 ±0.84.(Table 6.6). LDPL was found to have tapped density of 0.81 ±0.2 g/cc, angle of repose of 45.6 ± 3.1^{0}, Carr's compressibility index of 20.8 ±2.2 and residual moisture content of 7.3 ±0.98. Liposomal DPIs developed in the investigation were reported to have low density, good flowability and low residual moisture content.

It can be clearly observed from the above results the developed liposomal DPIs of ETP and DOC possessed volume mean diameter exceeding 5 μm with a tap density lesser than 0.4 g/cm^3 indicating the aerodynamic and light nature of DPI particles. Aerodynamic nature of these light and non massive particles renders the particles capable of escaping gravity induced and friction induced deposition in upper respiratory tract and can be targeted to deeper interiors of lungs and tumours prevailing therein.

L-Glycine was used as an anti adherent to reduce clumping of particle and thereby facilitates formation of aerodynamic, light particles with satisfactory flow properties and reduced probability of particle aggregation. The HA grafted drug(s) loaded liposomal DPIs developed in this investigation were found to exhibit tap density lesser than 0.4 g/cc, volume mean diameter exceeding 5μ and MMAD in range of 2-3 μ. This may be largely attributed to controlled particle size of liposomes and anti adherent nature of L-glycine. Non-reducing disaccharides are the most effective at protecting against drug leakage during freeze drying. Leopold et al, 1987 have suggested that sugars that tend to crystallize more readily may not be as effective and so lactose despite of being disaccharides are not effective compared to sucrose. It was also observed that sucrose as a carrier led to highest particle size (>11 μm) and even higher moisture content owing to its hygroscopic nature resulting in cohesiveness, poor flow and higher residual moisture content. The findings of the current investigation are in congruence with those reported by Chougule et al, 2007.

SEM reveals smooth and porous surface of LEDPIM and LDDPIM. Maximum FPF of 73.88± 1.17% was observed with LEDPIM as compared to LEDPIS of 50.76 ±3.16 and 67.23 ± 1.57 with LEDPIL. LEDPIM was observed to have 2.1 ± 0.31 and % dispersibility of 61.24 ± 5.44. Similarly, emitted dose was 67 ± 0.18 for LEDPIS and 85.12 ± 0.77 % for LEDPIM. Similarly, maximum %FPF in case of Docetaxel loaded liposomal DPIs was seen in case of LDDPIM. LDDPIM was observed to have %FPF of 70.41 ±1.46, % emitted dose of 78.08±0.16 and % dispersibility of 59.78 ± 2.16% as compared to 54.39 ±1.22 % FPF, 72.01 ±0.27 % emitted dose and 55.23 ± 1.45 % redispersibility of LDDPIS.

In vitro drug release study results indicated that DPIs of HA grafted liposomes of Etoposide and Docetaxel exhibited sustained release of Etoposide (97.3±1.3 % at the end of 48 h in case of LEDPIM as compared to > 93 % drug release in case of LEPL, Fig.7.3, Table 7.9) and Docetaxel (98.84 ±2.98 % at the end of 48 h in case of LDDPIM as compared to 97 % drug release within 3 h in case of LDPL, Fig. 7.4, Table 7.10) respectively from developed liposomal DPI formulations gearing a testimonial to the fact that liposomal DPI based delivery systems can help to retain drug for longer time in tumours in contrast to rapid wash out and removal of conventional anti cancer drug preparations.

It can be clearly observed in above results that LEDPIM and LDDPIM exhibited the best aerosol powder performance in this investigation in terms of emitted dose, MMAD, FPF, homogeneous size distribution among developed DPIs. The cutting edge performance of LEDPIM and LDDPIM over other DPI formulations may be attributed to aerodynamic, light and relatively larger particles, low tap density, good flow properties, less cohesiveness and lower moisture content.

7.9 REFERENCES

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