Chapter – 8

Stability studies of DPIs of HA grafted liposomal Etoposide and Docetaxel

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INTRODUCTION

One of the issues of major concern in liposomal products is poor stability of liposomal products during their shelf life. Product must exhibit a reasonably fair stability (atleast shelf life of one year) in order to render product cost effective and commercially feasible. Stability is a quintessential criterion that affects overall quality and acceptability of a product under question. (Talsma, 1993) Some of the major manifestations of instability reported in case of liposomal products are: drug leakage from vesicles to extra liposomal compartment, liposomal aggregation leading to clumping and formation of large particles. These physical changes largely affect the biological performance of the newly developed product. Besides these, lipid components of liposomes are likely to undergo hydrolysis and turn out rancid (Grit, 1993). However, preservation in dehydrated condition (lyophilized) largely averts this problem. Similarly, presence of unsaturated fatty acids can accelerate the oxidation of lipoidal components. Stability in broader sense refers to stability of chemical entity encapsulated within the vesicles, however, for optimum performance of a dosage form, it is essential to consider the formulation and process related factors that determine the stability and on reproducibility of in vivo drug behaviour. In the current investigation, DPIs of Hyaluronic acid grafted liposomes of Etoposide and Docetaxel were prepared using different cryoprotectants (Details discussed in Chapter 7: Formulation and optimization of HA grafted liposomal DPIs). Based on results of solid state characterization and in vitro lung deposition profile, DPIs of HA grafted liposomes of Etoposide and Docetaxel prepared using mannitol were found to be opitimized ones and hence, were observed for their stability aspects. In the following sections pertaining to stability aspects, we have focused on stability studies of DPIs of liposomal Etoposide and Docetaxel prepared using mannitol as a cryoprotectant. The stability protocol was designed as per ICH guidelines (Singh et al, 1999) for countries falling under zone III (hot, dry) and zone IV (very hot, humid).

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8.1 METHODOLOGY

Comparative stability studies were carried out of the potential liposomal DPI formulations at room condition (28°C±2°C, 60% ±5 RH/12 months) and at refrigerated conditions (5 ± 3°C/12 months) up to three months. Liposomal DPI formulations containing 550 µg BUD, 35 µg FRL and mixture of 300 µg ETP and 250 µg DOC were filled into gelatin capsule shells (Size 2). These prepared capsules were packed in HDPE bottles under nitrogen cover and the bottle was sealed with PVC coated aluminum foil. The bottles also contained silica bags as dehumectant and were resealed with flush of nitrogen after each sampling. Set of capsules from a batch were filled in the HDPE bottles for each condition.

The following parameters were considered as significant change:

5 % deviation in assay from initial value was considered as a significant change.

Sum total of degradation products exceeding acceptance criterion (Total related substances: Not More than 2%) and failure to meet acceptance criteria.

The liposomal DPIs formulations were also examined visually for the evidence of caking and discolouration. The content of the capsules are tested for assay, degradation, water content, PDR, emission and FPF. The stability results are summarized in Table 8.1 (LEDPIM), Fig. 8.1 (a), (b), (c), (d), (e) and Table 8.2 (LDDPIM) as well as Fig. 8.2 (a), (b), (c), (d) and (e).

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Table 8.1 Stability data of batch HA grafted ETP liposomal DPI formulation (LEDPIM)

Sampling Time (Month)	Description	Assay (%)	Volume Mean Diameter (VMD)μ*	Residual Moisture Content (%)*	Percentage Drug Retained (PDR)*	Fine Particle Fraction (FPF) %*
Initial (0)	White, free flowing powder	100 ± 1.5	5.9 ± 0.54	2.46 ±0.47	97.23 ± 0.54	76.75 ± 2.16
Refrigerated Conditions (5 ^{0}C ± 3^{0}C)						
1	White, free flowing powder	100 ± 2.5	6 ± 0.33	2.46 ± 0.38	97.0 ± 0.45	76.0 ± 0.56
3	White, free flowing powder	99.5 ± 2.0	6.2 ± 0.41	2.98 ± 0.42	96.40 ± 0.15	75.29 ± 0.77
6	White, free flowing powder	99 ± 1.5	6.4 ± 0.25	3.37 ± 0.56	96.11 ± 0.20	74.47 ± 1.98
12	White, free flowing powder	98 ± 2.0	6.9 ± 0.15	4.61 ± 0.28	94.72 ± 0.60	71.12 ± 2.11
Room condition (28°C±2°C, 60 ±5 RH)						
1	White, free flowing powder	99.5 ± 1.5	6.40 ± 0.47	2.91 ± 0.45	95.84 ± 0.30	75.0 ± 0.58
3	White, free flowing powder	98.25 ± 2.0	7.1 ± 0.41	3.6 ± 0.54	94.74 ± 0.2	71.18 ± 0.94
6	White, free flowing powder	96.00 ± 2.5	7.6 ± 0.25	4.75 ± 0.61	92.79 ± 0.20	68.34 ± 1.21
12	White powder with reduced flowability.	92.50 ± 2.0	8.80 ± 0.15	5.50 ± 0.33	91.43 ± 0.15	61.31 ± 1.97

***-Mean ±S.D. (n=3)**

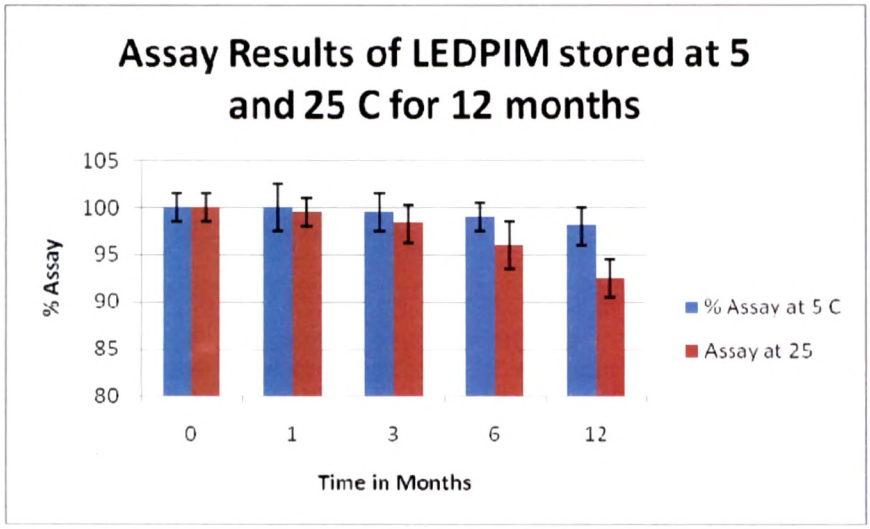

Fig 8.1(a)- Plot of % Assay Vs. Time in months for LEDPIM at 5 ± 3^{0}C and 25 ±2 ^{0} C/ 60 ± 5 % R.H. for 12 months.

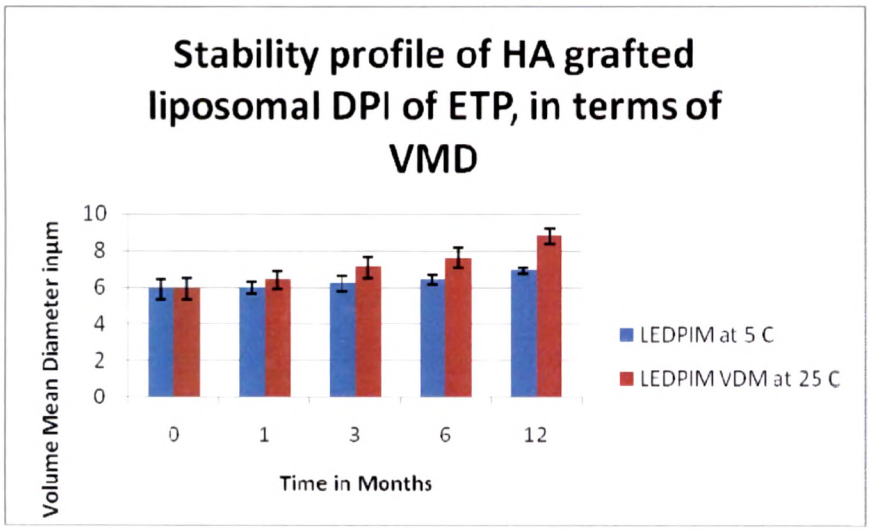

Fig 8.1 (b)- Plot of Volume Mean Diameter (VMD) Vs. Time in months for LEDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

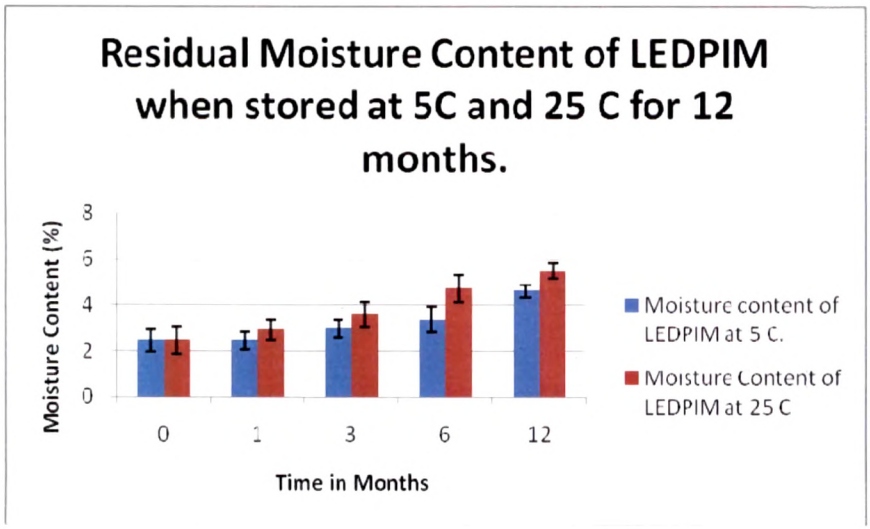

Fig 8.1 (c)- Plot of Residual Moisture Content Vs. Time in months for LEDPIM at 5 ^{0}C ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

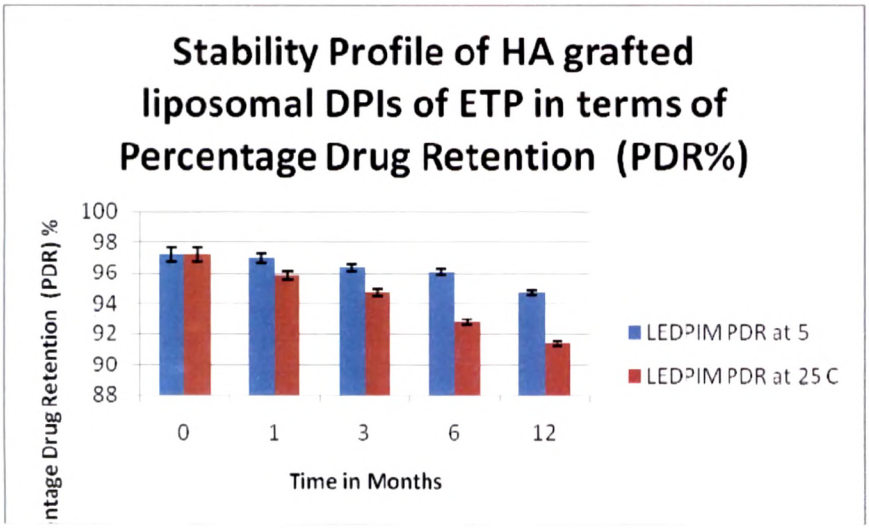

Fig 8.1 (d))-Plot of Percentage Drug Retention (PDR)Vs. Time in months for LEDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

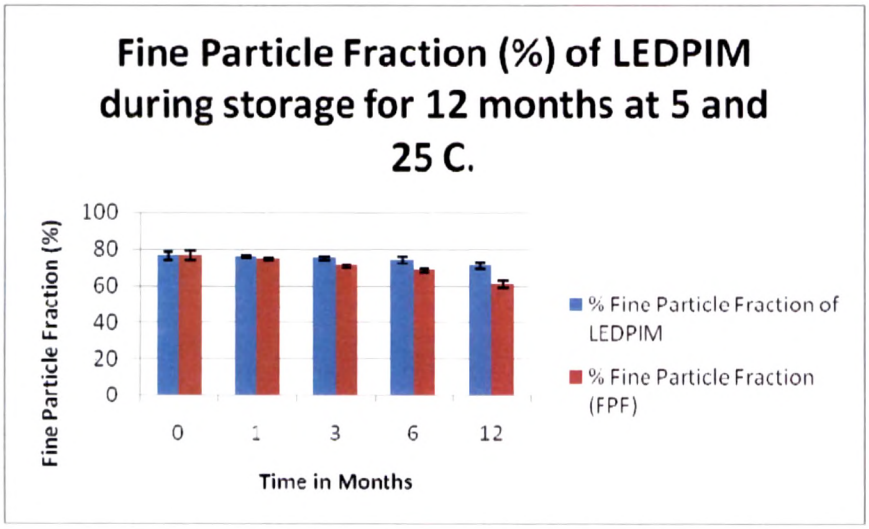

Fig 8.1(e) - Plot of Fine Particle Fraction (FPF) % Vs. Time in months for LEDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

Table 8.2 Stability data of batch HA grafted DOC liposomal DPI formulation (LDDPIM)

Sampling Time (Month)	Description	Assay (%)	Volume Mean Diameter (VMD)μ*	Residual Moisture Content (%)*	Percentage Drug Retained (PDR)*	Fine Particle Fraction (FPF) %*
Initial (0)	White, free flowing powder	100.5 ± 0.5	5.50 ± 0.31	2.46 ± 0.27	99.40 ± 0.45	70.41 ± 0.52
Refrigerated Conditions ($5^0C \pm 3^0C$)						
1	White, free flowing powder	100 ± 1.5	5.7 ± 0.45	2.46 ± 0.38	99.28 ± 0.55	70.19 ± 0.61
3	White, free flowing powder	99.5 ± 2.0	5.9 ± 0.61	2.98 ± 0.42	98.90 ± 0.60	69.21 ± 0.98
6	White, free flowing powder	99 ± 1.5	6.1 ± 0.26	3.37 ± 0.56	96.62 ± 0.50	67.31 ± 1.98
12	White, free flowing powder	98 ± 2.0	6.9 ± 0.61	4.61 ± 0.28	94.26 ± 0.80	62.29 ± 1.23
Room condition (28°C±2°C, 60 ±5 RH)						
1	White, free flowing powder	99.5 ± 1.5	6.0 ± 0.49	2.99 ± 0.31	98.81 ± 0.40	65.71 ± 2.11
3	White, free flowing powder	97.50 ± 2.0	6.7 ± 0.68	3.80 ± 0.34	96.78 ± 0.30	61.23 ± 2.41
6	White, free flowing powder	96.00 ± 2.5	7.80 ± 0.41	4.66 ± 0.29	93.14 ± 0.65	58.77 ± 1.91
12	White powder with reduced flowability.	92.50 ± 2.0	8.90 ± 0.65	5.71 ± 0.54	90.01 ± 0.70	52.79 ± 1.67

***-Mean ± S.D. (n=3)**

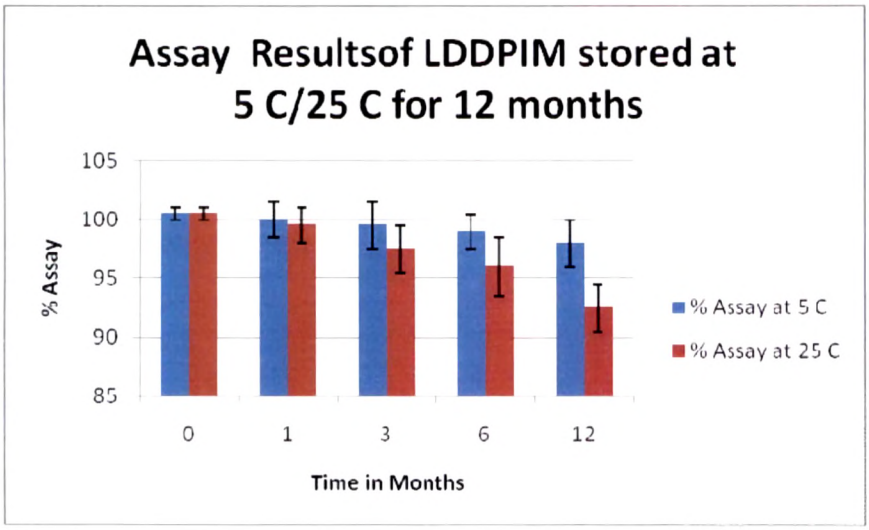

Fig 8.2 (a) - Plot of % Assay Vs. Time in months for LDDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

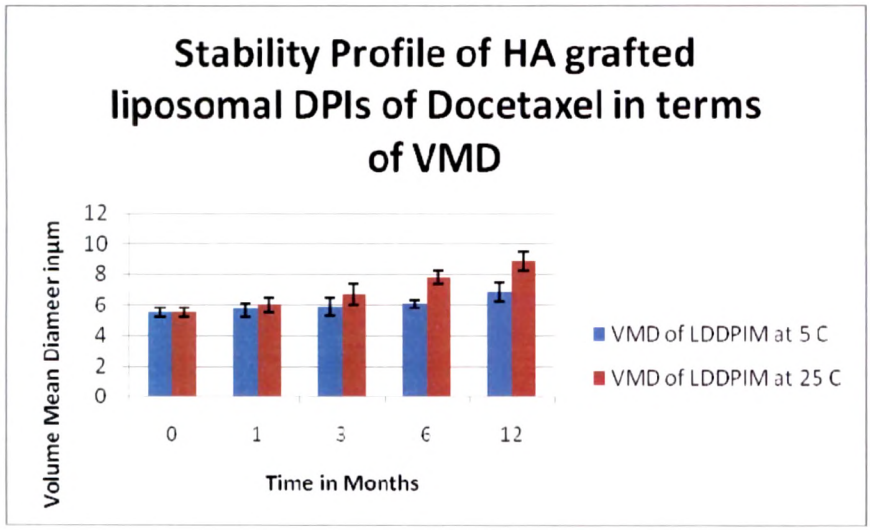

Fig 8.2 (b)- Plot of Volume Mean Diameter (VMD) Vs. Time in months for LDDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

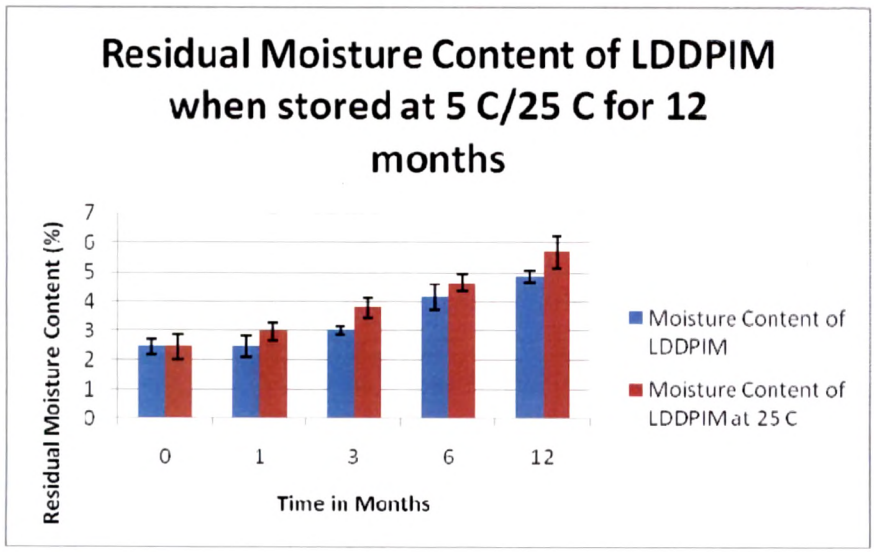

Fig 8.2 (c)- Plot of Residual Moisture Content (%) Vs. Time in months for LDDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

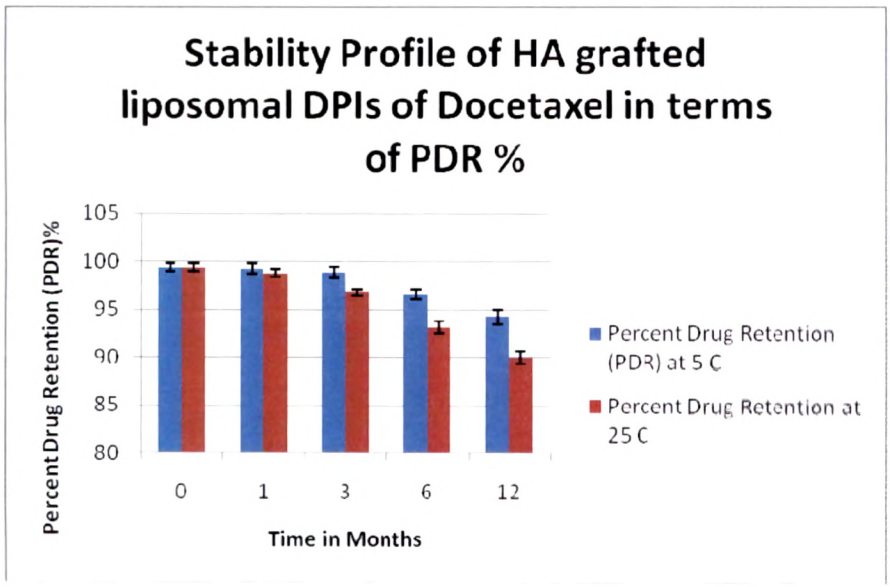

Fig 8.2 (d)- Plot of Percentage Drug Retention- PDR (%) Vs. Time in months for LDDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

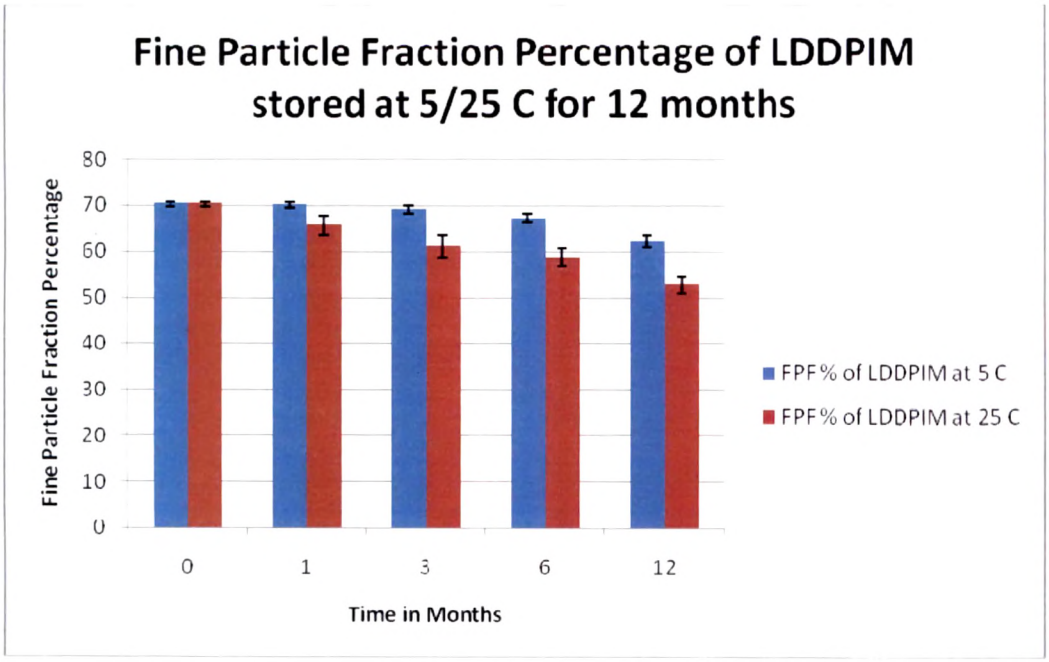

Fig 8.2 (e)- Plot of Fine Particle Fraction- FPF(%) Vs. Time in months for LDDPIM at 5 ± 3^{0}C and 25 ±2 ^{0}C/ 60 ± 5 % R.H. for 12 months.

8.2 DISCUSSION

Physical stability of liposomes is one of the most challenging issues in formulating a commercially feasible product. Liposomes should be stable for 1-2 years preferably at room temperature to be pharmaceutically acceptable with high drug retention within liposome and the particle size should be maintained during storage time, hence the drug leakage, particle size growth, *in-vitro* deposition characteristics, emission and the chemical stability of drugs were studied at room condition (28°C±3°C, 60% ±5 RH) and at refrigerated conditions (2-8°C) up to twelve months.

Liposomal DPIs did not exhibit any significant changes in their assay values indicating the stability of drug encapsulated within the liposomal compartment. There was no significant increase in particle size of DPI formulations stored and the latter were found to be stable till 6

months however, particle size increase was observed during 12 months storage period which may be attributed to aggregation of particles. HA by virtue of its negative charge, imparts negative charge to liposomal surface and prevents particulate aggregation by electrostatic repulsion, however, at room temperature there was significant increase in particle size indicating the increased tendency of particles to aggregate at elevated temperatures and hence, it is preferable to store liposomal DPIs under refrigerating conditions.

Low residual moisture content is essential for maintaining free flow characteristics of DPIs. Increase in moisture content leads to formation of sticky and cohesive mass obstructing the penetration of formulation to deeper peripheries of lungs when used by the patient. The presence of certain sugars has been shown to enhance stability. The best evidence available suggests that there is a direct interaction between the sugar and the polar head group of the phospholipid, the result of which is a depression of the transition temperature of the lipid and its maintenance in a fluid state even in the absence of water. (Crommelin, 1997, Bendas, 1998) However, HA being hygroscopic in nature tends to absorb moisture. This was evident in case DPIs stored under room conditions (higher relative humidity) where increase in residual moisture content of DPIs was observed. The increased moisture uptake during storage period may be attributed to hygroscopic nature of HA.

Similarly, liposomal DPI formulations stored at room temperature conditions exhibited reduction in percentage drug retention. Decrease in PDR with corresponding increase in temperature may be attributed to fluidization of phospholipid membranes of liposomes. With increase in temperature or at temperatures closer to phase transition temperatures of lipid components, rigidity of lipid membranes reduces resulting in increased fluidity through which drug tends to leak out. (Shah, 2004) In case of LDDPIM, there was remarkable decrease in percentage drug entrapment since Docetaxel tended to leak out from liposomal membranes to extra liposomal compartment and thus, exhibited lesser PDR as compared to that observed with LEDPIM.

No significant change in FPF % was observed in case of LEDPIM stored for 12 months at refrigerating condition; however, the one stored at room temperature exhibited significant reduction in FPF % which may be attributed to increased particle size due to aggregation and

cohesion induced by moisture uptake, thus reducing the effective particle fraction falling in range of 1-5 μm.

The DPI products did not exhibit any discolouration or change in organoleptic characters indicating fair stability of prepared DPIs, however, reduction in flow properties was observed which may be attributed to slight aggregation that occurred at higher temperature and increased cohesiveness due to moisture uptake under room temperature and humidity conditions.

On the whole, developed liposomal DPIs of Etoposide and Docetaxel were stable for 12 months when stored under refrigerating conditions on the whole and there was insignificant change in assay, PDR, residual moisture content, VMD, FPF and related parameters. Hence, one can arrive at a conclusion that in order to ensure maximum stability of liposomal DPIs, they ought to be stored under refrigerating conditions (2-8^{0}C).

8.3 REFERENCES

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