CHAPTER 5
STABILITY STUDIES

5. STABILITY STUDIES

5.1 Introduction

The physical and chemical properties of the formulation relevant to biological must be maintained during storage and distribution, therefore stability studies of formulation are required to be performed. Physical and chemical data are generated as a function of time and storage conditions (e.g., temperature and relative humidity [RH]). Stability testing provides evidence that the quality of a drug substance or drug product under the influence of various environmental factors changes with time.

5.1.1 Stability of liposome

Stability studies of liposome are carried out to evaluate the change in particle size, zeta potential, percent drug loading, chemical stability of phospholipids and drug over the period at different storage conditions. Change in the size of liposome can take place over the period of time and these changes can be the results of aggregation and fusion. Drug molecule can leak from the liposome which in turns reduces the entrapment efficiency. The leakage of the drug form the liposome depends upon liposome composition and physicochemical nature of the drug. Various methods have been used in improving the stability of liposome, like incorporating saturated phospholipids to avoid oxidation which is generally observed with unsaturated phospholipids, using charged phospholipids to reduce the possibility of aggregation and fusion, freeze drying of liposome in presence of cryoprotectanct to increase shelf life of liposome etc.

The optimized formulations were studied for their stability and their potentials to withstand the atmospheric/environmental changes. These formulations were subjected to stability testing by evaluating parameters like particle size, zeta potential and percent entrapment efficiency.

5.2 Experimental Methods

5.2.1 Liposome stability

Prepared liposomes (CL, SL and FT) were stored in Type-I amber colored glass vials. The samples were stored at 2-8°C (refrigeration condition) and at room temperature (30 ± 5°C). Sample from aqueous dispersion were withdrawn at regular interval and analyzed for drug stability, particle size, zeta potential (measured using Zeta sizer) and percent entrapment. The study was performed in triplicate and reported as mean of three batches of same composition. The results of stability studies are shown in Table 5.1.

Table 5.1 Stability data of liposomal dispersion

Stability conditions	Particle size (nm)			Zeta potential (mV)			%EE		
	CL	SL	FT	CL	SL	FT	CL	SL	FT
Initial	103.60± 4.58	120.30±6.46	98.43±4.18	4.71 ± 0.97	-7.33 ± 2.13	3.61 ± 1.31	74.83 ± 2.65	73.36± 2.34	75.16± 2.92
2-8 °C (Refrigerator conditions)									
1 Month	112.43± 6.53	131.28± 9.01	105.65± 5.49	4.21 ± 1.07	-6.57 ± 2.74	3.46 ± 1.04	74.26 ± 2.26	72.29 ± 2.52	74.43 ± 2.74
2 Months	116.24± 5.69	137.25± 6.36	109.83± 7.35	4.54 ± 1.12	-7.14 ± 1.84	3.78 ± 1.22	73.94 ± 1.96	72.05 ± 3.63	73.74 ± 2.43
3 Months	118.93± 8.21	141.86± 4.55	115.48± 4.49	3.84 ± 1.02	-7.46 ± 2.63	3.54 ± 1.63	72.78 ± 2.84	71.26 ± 2.36	72.57 ± 2.32
30 ± 5°C (Room temperature)									
1 Month	119.24± 8.14	136.94± 7.38	115.03± 6.48	4.94 ± 1.32	-6.33 ± 2.83	4.02 ± 1.92	68.53 ± 3.24	69.12 ± 4.12	67.36 ± 3.26
2 Months	122.56± 6.43	144.43± 6.43	119.35± 9.86	5.02 ± 1.41	-6.78 ± 1.95	3.73 ± 1.14	64.14 ± 2.48	63.46 ± 2.72	64.64 ± 2.93
3 Months	131.68± 7.34	155.35± 7.45	124.38± 6.84	4.36 ± 1.36	-6.14 ± 2.84	3.81 ± 0.97	60.93 ± 3.48	60.29 ± 2.64	61.46 ± 2.84

The % EE- percent entrapment efficiency. The values represents mean of three batches with ± SD

5.3 Results and Discussion

The stability of liposomes depends upon conditions like temperature/humidity and is affected by oxidation/hydrolysis etc. which leads to aggregating and leakage of the entrapped material from the vesicles and this greatly affect the shelf life of liposomes. Liposomal formulations have poor stability and do not meet the required standards for long term stability of pharmaceutical preparations if they are stored as aqueous dispersions. Decrease in the entrapment efficiency, fusion and aggregation are the major stability problems associated with liposomes preparation. Lyophilization is considered a promising means of extending the shelf-life of liposomes. However, both freezing and drying can induce structural and functional damage to liposomes. Sugars are frequently used as the cryoprotectants in lyophilization for stability of liposomes (Lu and Hickey, 2005).

Here the liposomal aqueous dispersion were evaluated for the effect of temperature on the particle size, zeta potential and percent entrapment efficiency of the liposomes and for stability over the period of 3 months at refrigeration (2-8°C) and at room temperature (30±5°C).

Liposomal formulations were stable when stored at 2-8°C as aqueous dispersion, whereas aqueous dispersion stored at 30±5°C showed increase in particle size after 2 month, compared to the initial particle size (Table4.1). Saturated phospholipid (HSPC) used in the preparation of liposome and also due to higher negative zeta potential which decreases Vander Waals interaction (which is a major contributor to aggregation of electrostatically neutral complexes) of the liposome, ultimately preventing liposomes from fusion and aggregation (Maitani et al., 2008). The entrapment efficiency of liposomal dispersion lowered with time at all storage condition but maximum decrease in entrapment efficiency was observed for aqueous dispersion at 30±5°C storage condition (Table 5.1).

5.4 Conclusion

Hence, it can be concluded that liposomal dispersion can be stored at 2-8°C as aqueous dispersion for 3 month are stable. Liposomal dispersion stored at 30±5°C shows as a decrease in entrapment efficiency and increase in particle size.

References

1. Lu D, Hickey AJ. Liposomal Dry Powders as Aerosols for Pulmonary Delivery of Proteins. AAPS Pharm Sci Tech. 2005; 6(4): E641-E648.
2. Maitani Y, Aso Y, Yamada A, Yoshioka S. Effect of sugars on storage stability of lyophilized liposome/DNA complexes with high transfection efficiency. Int. J. Pharm. 2008; 356: 69–75.