Chapter 11

STABILITY STUDY

11. 1 Introduction

Stability testing is the primary tool used to assess expiration dating and storage conditions for pharmaceutical products. Protocols used for stability testing are based on the recommendations of the International Conference on Harmonization (ICH). Stability studies are linked to the establishment and assurance of safety, quality and efficacy of the drug product from early phase development through the lifecycle of the drug product. Liposomes have been extensively investigated for drug delivery, drug targeting, controlled release and enhancing solubility. However the major limitation in the widespread use of this versatile drug delivery system is its instability. If liposomes are developed to enter market as products, they must be stable during the storage period, and remain at the optimized size and intact before reaching their targeted tissues to produce the desired effect. [1]

Physical processes such as aggregation/flocculation and fusion/coalescence that affect the shelf life of liposomes can result in loss of liposome associated drug and changes in size. Aggregation is the formation of larger units of liposomal material; these units are still composed of individual liposomes. However, the presence of aggregation can accelerate the process of coalescence of liposomes, which indicates that new colloidal structures are formed. As coalescence is an irreversible process; the original liposomes cannot be retrieved. A colloidal dispersion is often thermodynamically unstable. [2] Hence, it is crucial to assess the stability of liposomal formulation upon storage.

11.2 Materials and Equipment

Materials

Methanol and Chloroform (A. R. grade) was purchased from Merck, Mumbai, India. Sodium Hydroxide, Potassium Hydroxide, Calcium Hydroxide, Hydrochloric acid and Acetic acid were obtained from Loba Chemie, Mumbai, India. Bovine Serum Albumin, Lactic acid and Glycerol were procured from Merck Specialities Pvt. Ltd. Mumbai, India. Sodium Chloride, Urea and Glucose were purchased from Qualigens Fine Chemicals, Mumbai, India. Distilled water used in the study was filtered using 0.22 micron nylon

filter, Nylon N66 membrane filters 47 mm, Rankem, India. All other chemicals used were obtained from authentic source and were of Analytical Reagent grade.

Equipment

- Analytical Weighing Balance (ATX 224, Shimadzu, Japan)
- Vortex Mixer (Spinix-Vortex Shaker, Tarsons, India)
- Ultrasonic Bath Sonicator (Ultrasonics Selec, Vetra, Italy)
- UV-Visible Spectrophotometer (UV1800, Shimadzu, Japan)
- Magnetic Stirrer (Remi Instruments, Mumbai, India)
- Cooling centrifuge (Remi Equipments, Mumbai, India)
- Malvern Zetasizer (NanoZS, Malvern Instruments, UK)

11.3 Method

Short-term accelerated stability studies were performed according to the ICH Q1A (R2) guidelines for all the three liposomal formulations as well as the Intra Vaginal Rod inserts. The vesicle size and entrapment efficiency are sensible indicators of the stability of liposomal dispersions. While the Intra Vaginal Rod inserts were examined for physical appearance and In Vitro drug release. All the three liposomal formulations and IVR rod inserts were stored in transparent glass vials at 2-8 ℃ (refrigerated) and 25 -30 ℃ (room temperature) for 3 months. At specific time intervals of 1, 2, and 3 months, the samples were taken, and their physical appearance was examined. In addition, the mean vesicle size and entrapment efficiency of all the three optimized liposomal formulations were measured by method already described in chapter 5. While for IVR rod inserts, in vitro drug release was determined by the method described in chapter 6.

11.4 Results and Discussion

Stability study results of all the three optimized liposomal formulations for vesicle size and entrapment efficiency are given in Table 11.1, 11.2 and 11.3 It was observed that all the three liposomal dispersions showed physical stability over the period of 3 months at

2-8 ℃. While the vesicle size and entrapment efficiency were found to change significantly upon storage at room temperature 25-30 ℃ for beyond a month.

The entrapment efficiency at room temperature was found to decrease during storage and the vesicle size increased above the optimized range for all three liposomal dispersion. The increase in the vesicle size upon storage at room temperature may be attributed to the aggregation of the vesicles. While the decreased entrapment efficiency at room temperature can be because of the oxidation and hydrolysis of lipids that may lead to the appearance of short-chain lipids and formation of soluble derivatives in the membrane, resulting in the decrease of the quality of liposomal bilayer structure leading to leakage of the entrapped drugs. [3] Thus, it was concluded that the optimum temperature condition for storage of the liposomal formulations would be refrigerated condition (2-8 ℃).

Table 11.1 Stability data of RLX loaded optimized Liposomes

Storage condition	**Vesicle size (nm)**		**% EE**	
	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)
Initial	122.1±1.2	122.1±1.2	90.96±1.4	90.96±1.4
1 month	127.45±1.4	122.98±1.0	86.14±1.2	89.98±1.2
2 months	135.56±2.1	123.43±1.1	79.23±1.1	89.45±1.1
3 months	148.67±2.5	123.79±1.4	72.46±1.3	89.32±1.0

*Experiment was done in triplicate (n=3)

Table 11.2 Stability data of LA loaded optimized Liposomes

Storage condition	**Vesicle size (nm)**		**% EE**	
	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)
Initial	432.5±2.1	432.55±2.1	74.63±1.1	74.63±1.1
1 month	438.16±1.5	432.84±1.1	72.14±1.1	73.93±1.2
2 months	446.67±1.1	433.27±1.3	66.76±1.3	73.11±1.4
3 months	457.34±1.2	433.87±1.2	59.19±1.2	72.25±1.5

*Experiment was done in triplicate (n=3)

Table 11.3 Stability data of dual drug (RLX-LA) optimized loaded Liposomes

Storage condition	Vesicle size (nm)		% EE			
	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)	Room temp. (25-30 ºC)		Refrigerated temp. (2-8 ºC)	
			RLX	LA	RLX	LA
Initial	354.4±1.0	354.40±1.0	90.91±1.1	74.30±1.0	90.91±1.1	74.30±1.0
1 month	359.12±1.2	353.15±1.2	85.34±1.3	71.44±1.4	89.45±1.2	73.87±1.5
2 months	365.45±1.1	352.89±1.3	78.67±1.2	67.56±1.1	89.14±1.1	73.24±1.2
3 months	372.98±1.4	352.11±1.1	69.87±1.4	58.98±1.2	88.97±1.3	72.96±1.4

*Experiment was done in triplicate (n=3)

The stability study results for IVRs are given in Table 11.4 and 11.5. Upon storage at two different conditions, effect on drug release was evaluated. Appearance of all the IVRs i.e. RLX liposomes loaded, LA liposomes loaded and dual drug (RLX-LA) loaded liposomes remained the same. It was yellowish white in color. It was noticed that there was no significant change in the drug release profile of IVRs after 2-month storage at room temperature however after 3 months, the release rate was found to be increased but the difference was not significant. Similarly at refrigerated conditions, the release profile remained the same throughout with no change. This is due to the freeze -dried form of liposomes present in the rods, which prevents any changes in the release profile. However, the optimum storage conditions for the IVR would be 2-8 ºC (refrigeration) to maintain the long-term stability of the liposomal formulation incorporated inside the rod inserts.

Table 11.4 Stability data of IVR loaded with optimized RLX-Liposomes and optimized LA-Liposomes

Storage condition	% Drug release after 144 hours RLX-IVR		% Drug release after 144 hours LA-IVR	
	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)	Room temp. (25-30 ºC)	Refrigerated temp. (2-8 ºC)
Initial	99.66±1.1	97.27±1.1	99.31±1.2	99.31±1.2
1 month	97.85±1.4	97.12±1.5	99.54±1.2	99.58±1.5
2 months	97.92±1.5	97.56±1.1	99.93±1.1	99.89±1.1
3 months	99.98±1.1	99.97±1.2	99.96±1.3	99.95±1.0

*Experiment was done in triplicate (n=3)

Table 11.5 Stability data of IVR loaded with optimized dual drug (RLX-LA) loaded Liposomes

Storage condition	**% Drug release after 144 hours RLX-IVR**		**% Drug release after 144 hours LA-IVR**	
	Room temp. (25-30 ^{o}C)	Refrigerated temp. (2-8 ^{o}C)	Room temp. (25-30 ^{o}C)	Refrigerated temp. (2-8 ^{o}C)
Initial	99.66±1.1	99.66±1.1	99.98±1.2	99.98±1.2
1 month	99.71±1.4	99.75±1.5	100.02±1.2	100.03±1.0
2 months	99.75±1.5	99.86±1.1	100.12±1.1	100.07±1.1
3 months	99.90±1.2	99.95±1.2	100.15±1.3	100.10±1.0

*Experiment was done in triplicate (n=3)

Thus, the developed liposomal formulations as well as the IVRs were found to be acceptably stable upon storage at Refrigerated temperature (2-8 ^{o}C) with negligible changes in the vesicle size, entrapment efficiency and drug release.

11.5 References

1) Yadav, A., Murthy, M.S., Shete, A.S. and Sakhare, S., 2011. Stability aspects of liposomes. Indian Journal Of Pharmaceutical Education And Research, 45(4), pp.402-413.

2) Takeuchi, H., Yamamoto, H., Toyoda, T., Toyobuku, H., Hino, T. and Kawashima, Y., 1998. Physical stability of size controlled small unilameller liposomes coated with a modified polyvinyl alcohol. International Journal of Pharmaceutics, 164 (1), pp.103-111.

3) Briuglia, M.L., Rotella, C., McFarlane, A. and Lamprou, D.A., 2015. Influence of cholesterol on liposome stability and on in vitro drug release. Drug delivery and translational research, 5(3), pp.231-242.