CHAPTER 6

STABILITY STUDIES

Stability Studies

6.1 INTRODUCTION

For liposomal products an attention has been focused on two processes affecting the quality and therefore acceptability of liposomes (Talsma and Crommelin, 1992). Especially, with liposomal product to see the market it should stable during the shelf life (storage or transport). In general, a shelf life of at least one year is a minimum prerequisite criterion for a commercial product First leakage of drug from the vesicles may take place into the extra liposomal compartment. Secondly, there is a possibility of liposomal aggregation and/or fusion, which leads to formation of larger particles (Slabbert et al., 2011; Fang et al., 1997; Cliff et al., 1992; Grit and Crommelin, 1992). These parameters will alter the *in vivo* fate, affecting therapeutic index of the drug. Hydrolysis of phospholipids is one of the parameters like to cause the formation of fatty acids and lysophopholipids (Grit and Crommelin, 1993; Mowri et al., 1984). Although under dehydrated storage, there is least possibility of the formulation to encounter hydrolytic degradation. Another aspect to be considered is liposome oxidation (Frokjaer et al., 1984). Stability is considered as chemical stability of drug substance in a dosage form. However, the performance of liposomal formulation is not only dependent upon the content of the drug substance, but also dependent on reproducible *in vivo* performance of the formulations. Formulations under stability studies were considered chemically stable by evaluating the drug leakage from liposomes. The stability protocol was designed as per ICH guidelines (Singh, 1999) for countries falling under zone III (hot, dry) and zone IV (very hot, humid) (US FDA, 2002).

6.2 METHOD

Comparative stability studies were carried out of the potential liposomal formulations at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (5°C ± 3°C) up to twelve months. Lyophilized liposomal formulations containing 0.2 mg RGZ and 0.2 mg CDS were filled into amber color glass vials, purged with nitrogen, sealed and stored at the above mentioned condition. At each sampling time different vial is used for the stability testing (Bhalerao and Raje, 2003; Manosroi et al., 2004; Manosroi et al., 2002; Yang et al., 2007; Anderson and Omri, 2004; Changsan et al., 2009; Winterhalter and Lasic, 1993; Ugwu et al., 2005).

The liposomal formulations were examined visually for the evidence of discoloration. The content of the vial are tested for percentage drug retention (PDR), particle size, zeta-potential, assay and water content. The stability results are summarized in Tables 6.1 (RGZ) and Table 6.2 (CDS).

6.3 RESULTS AND DISCUSSION

The physical stability of liposomes is one of the biggest obstacles in formulation commercially viable product (Fildes, 1981). 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, change in zeta-potential and the chemical stability of drugs were studied at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (5°C ± 3°C) up to twelve months. No significant differences were found in all above mentioned parameters at both conditions.

6.3.1 Stability testing of RGZ liposomal formulation

The stability testing of prepared RGZ liposomal formulation was performed at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (5°C ± 3°C) up to twelve months and the effect on various parameters was studied and reported below.

Table 6.1 Stability testing data of RGZ liposomal formulation

Sampling time (Month)	Description	Assay (%)	Percent drug retained	Water content (%)	Particle size [Z-Average (d.nm)]	Zeta potential (mV)
Initial	White powder	101.16 ± 2.27	101.55 ± 3.21	3.1 ± 0.21	180.1 ± 2.93	-21.9 ± 1.33
Accelerated condition (25°C ± 2°C, 60% RH ± 5% RH)						
1	White powder	101.08 ± 2.45	100.46 ± 3.47	3.2 ± 0.18	183.4 ± 2.74	-21.6 ± 1.27
2	White powder	101.32 ± 1.98	99.19 ± 2.87	3.2 ± 0.19	186.2 ± 2.22	-20.8 ± 1.43
3	White powder	100.73 ± 3.03	98.77 ± 2.63	3.3 ± 0.21	188.7 ± 3.19	-20.9 ± 1.51
6	White powder	99.79 ± 3.28	97.66 ± 3.07	3.4 ± 0.24	194.3 ± 3.35	-19.7 ± 1.20
Long-term conditions (5°C ± 3°C)						
1	White powder	101.77 ± 2.83	101.81 ± 2.26	3.1 ± 0.19	180.4 ± 3.02	-22.1 ± 1.21
2	White powder	101.43 ± 2.42	101.07 ± 3.09	3.1 ± 0.22	179.2 ± 3.11	-21.8 ± 1.49
3	White powder	101.09 ± 3.11	101.24 ± 3.13	3.1 ± 0.22	183.3 ± 2.95	-21.3 ± 1.46
6	White powder	101.82 ± 2.07	100.92 ± 3.42	3.2 ± 0.28	182.4 ± 2.84	-21.1 ± 1.13
9	White powder	100.68 ± 1.84	100.76 ± 2.86	3.2 ± 0.29	184.6 ± 2.50	-21.3 ± 1.59
12	White powder	100.31 ± 3.21	100.01 ± 2.29	3.2 ± 0.24	186.2 ± 3.14	-20.7 ± 1.09

6.3.2 Stability testing of CDS liposomal formulation

The stability testing of prepared CDS liposomal formulation was performed at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (5°C ± 3°C) up to twelve months and the effect on various parameters was studied and reported below.

Table 6.2 Stability testing data of CDS liposomal formulation

Sampling time (Month)	Description	Assay (%)	Percent drug retained	Water content (%)	Particle size [Z-Average (d.nm)]	Zeta potential (mV)
Initial	White powder	99.82 ± 2.53	98.93 ± 3.38	2.7 ± 0.19	**184.6 ± 3.01**	**-29.3 ± 1.41**
Accelerated condition (25°C ± 2°C, 60% RH ± 5% RH)						
1	White powder	99.98 ± 2.21	98.12 ± 3.19	2.7 ± 0.23	185.1 ± 2.69	-28.5 ± 1.38
2	White powder	99.76 ± 2.33	96.74 ± 3.26	2.7 ± 0.23	188.4 ± 2.09	-27.6 ± 1.26
3	White powder	99.41 ± 2.72	96.39 ± 2.87	2.8 ± 0.19	190.3 ± 2.84	-27.3 ± 2.01
6	White powder	98.63 ± 1.97	95.28 ± 2.82	2.9 ± 0.20	196.8 ± 3.09	-26.2 ± 1.42
Long-term conditions (5°C ± 3°C)						
1	White powder	99.54 ± 3.02	98.47 ± 2.71	2.7 ± 0.18	183.9 ± 3.25	-29.2 ± 1.19
2	White powder	99.61 ± 2.21	98.59 ± 3.31	2.7 ± 0.25	184.7 ± 2.88	-29.6 ± 1.44
3	White powder	99.71 ± 2.95	98.38 ± 3.47	2.7 ± 0.23	185.5 ± 2.61	-28.1 ± 1.37
6	White powder	99.34 ± 3.16	98.16 ± 2.56	2.7 ± 0.31	187.2 ± 3.37	-27.6 ± 1.33
9	White powder	99.28 ± 2.78	97.87 ± 2.72	2.7 ± 0.25	189.9 ± 3.00	-27.4 ± 1.72
12	White powder	98.94 ± 2.45	97.12 ± 3.01	2.8 ± 0.27	193.1 ± 2.98	-26.9 ± 1.21

6.4 CONCLUSION

The decrease in drug assay, percentage drug retained, and zeta-potential and increase in water content, particle size were observed at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (5°C ± 3°C) up to twelve months for both RGZ and CDS liposomal formulation but the changes were statistically insignificant. Hence, both the formulations were considered as stable and were selected for further studies.

6.5 REFERENCES

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