Chapter 11

Stability Studies

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Stability study of any formulation on storage is necessary as it reflects whether the desirable properties of the formulation are retained on storage (1, 2). These desirable properties include integrity of lipid vesicles and size distribution of particles. Upon storage, liposomes are susceptible to many physical changes i.e. lipid particles may undergo fusion and aggregation leading to increase in particle size of liposomes. Also there may occur loss of integrity of liposomes and subsequently leakage of encapsulated drug may take place (3, 4). Liposomal formulations are not stable enough in an aqueous dispersion form. So, to increase their stability the liposomal formulations are freeze-dried (lyophilized). However, during lyophilization the liposomal formulation may undergo aforementioned physical changes. To avoid such changes different lyoprotectants like sucrose, mannitol, glycerol, trehalose, povidone, dextran etc. can be used which maintain the product in a good state (5, 6). The physical testing of such product should be carried out to check whether any changes take place in the liposomal product in terms of its particle size and entrapment efficiency. So, after storage period, the liposomal formulation, on rehydration, should retain the same characteristics it possessed before lyophilization. For liposomal products an attention has been focused on two processes affecting the quality and therefore acceptability of liposomes (7). First leakage of entrapped molecules 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 (8-11). Although under dehydrated storage, there is least possibility of the formulation to encounter hydrolytic degradation. Another aspect to be considered is liposome oxidation (12)..

As per the ICH stability study guideline Q1A (R2), stability studies should be performed on a drug product intended for storage in refrigerator at following storage conditions. (Table 5.4) The stability protocol was designed as per ICH guidelines (13) for countries falling under zone III (hot, dry) and zone IV (very hot, humid) (14); however, only short term studies for 3 months storage period were performed for having the idea of the stability of the product..

Table 11.1 Stability Testing Conditions for Drug Product Intended for Storage in Refrigerator as per ICH Guideline Q1A(R2).

Study	Storage condition	Time period for which study should be carried out
Long term.	.5°C ± 3°C.	.12 months.
Accelerated	.25°C ± 2°C/60% RH ± 5% RH.	.6 months.

Any "significant change" for a drug product is defined as:

1. A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;
2. Any degradation product's exceeding its acceptance criterion;
3. Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions;
4. Failure to meet the acceptance criterion for pH;
5. Failure to meet the acceptance criteria for dissolution for 12 dosage units..

11.1.1 Method

.Comparative stability studies were carried out of the potential lipoplex formulations at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for three months and at long-term conditions (2-8°C) up to three months. Lyophilized formulations in Type I tubular glass vials were sealed with chlorobutyl rubber stoppers and sealed with aluminum seals. Sealed vials were stored at above mentioned condition (15-22). At each sampling point different vials were used for the stability testing. The lipoplex formulations were examined visually for the evidence of discoloration. The content of the vials were tested for percentage pDNA complexation, assay, particle size, zeta-potential, assay and water content.

11.1.2 Results and discussion

Lipoplex formulations must show physical stability in order to produce a commercially viable product (23). Preferable stability of formulation up to 1 to 2 years at

storage conditions i.e. room temperature condition or at refrigerated condition is required for a pharmaceutically acceptable liposomal product. In order to make the formulation survive these long stability periods on shelf, lyophilization becomes a primary resort for stabilizing the liposomal product. However, this doesn't eliminate the requirements for the real-time stability monitoring as the physicochemical properties of the formulations are still prone to change on storage. To evaluate the stability of the pDNA lipoplex formulations, lyophilized liposomal formulations were evaluated for changes in particle size, zeta potential, pDNA assay and complexation efficiency were determined. Results of the study are tabulated in **Table 11.2**. Due to similar compositions of the lipoplexes except for the modified lipid, the stability data can be extended to have an idea on the stability of the other lipoplexes as well.

During stability monitoring, no significant differences ($p > 0.05$) were found in all above-mentioned parameters at refrigerated condition. Lyophilize formulations maintained their physical integrity and were observed as white porous cakes. Assay of the formulations stored at both conditions at each time point was between the range of 95-105% of initial levels which was acceptable. There was no significant change ($p>0.05$) in particle size and zeta potential after storage period at both conditions. Water content of the lyophilized cakes was not affected during the storage period ($p< 0.05$). Stability studies at accelerated and refrigerated conditions demonstrate that the product was stable at both conditions for a period of 3 months and suggest that the product will be stable for longer periods at refrigerated conditions.

Table 11.2 Stability Testing Data of gHDSPE lipoplexes

Storage condition	Time (Month)	Description	Assay (%)	Complexation efficiency (%)	Water content (%)	Particle size (nm)	Mean Polydispersity index	Zeta potential (mV)
Initial (Before lyophilization)		NA	101.23±2.54	100.52±1.89	NA	136.4±5.7	0.262	26.4±1.2
Initial (After lyophilization)		White lyophilized cake	99.42±2.04	99.25±2.15	1.26±0.30	140.7±4.2	0.271	24.5±1.2
2-8°C	1	White lyophilized cake	100.16±1.58	98.69±1.95	1.34±0.27	141.5±5.1	0.249	21.3±1.4
2-8°C	2	White lyophilized cake	98.56±1.24	99.15±2.10	1.16±0.15	143.4±4.1	0.272	20.9±0.9
2-8°C	3	White lyophilized cake	99.48±1.85	98.88±1.19	1.39±0.21	142.5±3.8	0.266	19.4±1.6
25°C/60% RH	1	White lyophilized cake	98.75±1.48	99.56±2.65	1.35±0.25	159.9±4.9	0.232	16.2±1.1
25°C/60% RH	2	White lyophilized cake	99.71±2.03	99.12±2.36	1.44±0.26	176.5±4.5	0.280	10.4±1.5
25°C/60% RH	3	White lyophilized cake	98.48±1.65	98.78±1.56	1.52±0.34	190.4±2.9	0.354	7.7±1.4

*Experiments were performed in triplicate

11.2 References

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