# CHAPTER 6. STABILITY STUDIES

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## 6. INTRODUCTION

Stringent requirements have been laid forward by regulatory authorities for stability of nanoparticulate drug delivery systems like liposomes. Hence, it is crucial to determine stability of all nanoparticulate systems designed for delivery of therapeutics. Additionally, developed liposomal formulation being a carrier for hydrophilic drug, there are chances that equilibrium may get shifted and formulation may get destabilized over time on exposure to humidity and temperature. Liposome systems have been reported to show various physicochemical changes on storage. Such changes include liposomal aggregation, fusion, loss of drug, etc. (1-4). These parameters will affect the *in vivo* performance of the formulation (8, 9). Additionally, phospholipids may undergo hydrolysis reaction forming fatty acids and lysophospholipids (5, 6). However, under dried state, there is least possibility for such degradation, but, there are still chances of hydrolysis due to residual water content remaining in lyophilized cakes and also under humid conditions and temperature. Another aspect of stability of liposomes is oxidation of lipids (7). These changes may lead to structural integrity problems in liposomes and this might cause release of entrapped drug. Thus these effects induce time dependent changes in desired properties of formulation during storage, therefore real time stability studies are potential tools to get an idea of any such possibility.

Optimized liposomal formulation was lyophilized for better stability. However, the lyophilized formulations should retain their original characteristics on stability. Hence, stability studies were performed on lyophilized liposomal formulations containing drug by entrapment efficiency, particle size and change in water content of the formulation. Stability studies were performed at two conditions i.e. 2-8°C and 25°C as specified by ICH guidelines for products to be stored in refrigerator.

### 6.1 Method

Liposomal formulation was evaluated for long term stability for 3 months at accelerated conditions of 25°C ± 2°C, 60% RH ± 5% RH and at 5°C ± 3°C. Liposomal formulations were filled into type-1 tubular glass vials, purged with nitrogen, sealed and stored at the above mentioned condition [11-17]. At each sampling time different vial was used for the stability testing. Lyophilized formulation was examined visually for any discoloration or shrinkage/collapse of lyophilized cake. The content of the vial were examined for moisture content using Karl-Fischer titration. Weighed quantity of formulation was reconstituted

appropriately with water for injection and used for analysis of entrapment efficiency, particle size and zeta potential as described in previous sections. The stability results are summarized in **Table 6.1.**

## 6.3 Results and Discussion

The physical stability of liposomes is one of the biggest obstacles in formulation commercially viable product [19]. Liposomes should be stable for 1-2 years preferably at room temperature or refrigerated condition, whichever is storage temperature, to be pharmaceutically acceptable with high entrapment retention within liposome and the particle size should be maintained during storage time, hence the drug leakage, particle size growth and change in zeta-potential were studied 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. No significant differences ($p>0.05$) were found in all above mentioned parameters at refrigerated condition.

### 6.3.1 Stability Testing of RGD-grafted optimized Liposomes

The stability testing of prepared RGD- grafted optimized liposomes (3%) was performed 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 and the effect on various parameters was studied. Results of the study are reported below (**Table 6.1**). At both accelerated and refrigerated conditions, Assay and drug entrapment values were found to be within range (90-110% of initial) and change was non-significant ($p>0.05$). There was no significant increase ($p>0.05$) in particle size after three month at both conditions and same results were shown by zeta potential values. Water content was increased to a significant extent ($p<0.05$) at accelerated condition while refrigerated condition maintained the water content value even after three months of storage. Thus, formulation is stable at both conditions after three months.

Visual examination of the formulations stored at both stability conditions showed no evidence of any physical instability. There was no significant particle size increase on storage either at accelerated condition or at refrigerator condition. However, there was a marginal increase in the polydispersity index at accelerated stability condition. Our results conform to the results obtained by other authors who have attributed to the retained particle size characteristics with the lyophilization of the formulations with suitable cryoprotectant (10). Zeta potential was also recorded to see whether there was change in surface charge properties as well as to have idea about the stability of reconstituted formulations. Studies revealed that, zeta potential was

maintained close to initial zeta potential at both storage conditions and this depicts that there was no drastic changes happened in the formulation during the stability.

Table 6 1Stability Testing Data of RGD-grafted optimized Liposomes (3%)

| Sampling time (Month) | Description | Assay (%) | GEM entrapment (%) | Water content (%) | Particle size (d.nm) | Particle distribution Index | Zeta potential (mV) |
|---|---|---|---|---|---|---|---|
| Initial | White lyophilized cake | 101.27 ± 2.14 | 62.06 ±1.54 | 1.79 ±0.20 | 147 ±2 | 0.155 | -55.26 ±0.55 |
| **Accelerated condition (25°C ± 2°C, 60% RH ± 5% RH)** | | | | | | | |
| 1 | White lyophilized cake | 101.05 ± 1.25 | 61.83 ±2.10 | 1.96 ±0.15 | 149 ±2 | 0.170 | -55.16 ±0.52 |
| 2 | White lyophilized cake | 99.02 ± 2.18 | 59.73 ±1.52 | 2.71 ±0.34 | 156 ±3 | 0.201 | -54.88 ±1.13 |
| 3 | White lyophilized cake | 98.13 ± 1.04 | 57.91 ±1.45 | 3.19 ±0.54 | 171 ±4 | 0.186 | -53.98 ±1.11 |
| **Long-term conditions (2-8°C)** | | | | | | | |
| 1 | White lyophilized cake | 101.12 ± 2.63 | 63.06 ±1.49 | 1.81 ±0.15 | 148 ±2 | 0.169 | -55.73 ±1.01 |
| 2 | White lyophilized cake | 100.24 ± 2.02 | 62.05 ±1.50 | 1.91 ±0.32 | 152 ±4 | 0.172 | -54.85 ±1.62 |
| 3 | White lyophilized cake | 102.15 ± 1.20 | 61.32 ±1.75 | 2.09 ±0.50 | 164 ±2 | 0.161 | -55.48 ±1.01 |

Moisture content is one of the important parameters that can affect the stability of dry powders. Residual moisture below 1% in lyophilized powders is considered better for storage stability (11). However, higher moisture content can lead to aggregation in lyophilized formulations during storage (12). Such aggregation of particles can lead to increased particle size which can result in suboptimal and inconsistent dose delivery, injectability and syringeability issues upon formulation delivery. It has also been reported that moisture content can lead to destabilization of nano systems due to water induced crystallization of carbohydrates when they are held above Tg of the system (13, 14). Water induces shift in the Tg of the system below the temperature of storage augmenting the crystallization processes (14). Hence, moisture content analysis was performed using Karl-Fisher titration. The analysis showed that moisture contents were significantly increased for all formulations at accelerated

conditions reaching more than 2% level; however, on storage at refrigerated conditions lyophilized cakes maintained moisture content comparable to initial. Higher moisture contents at accelerated conditions were in concordance with other studies (15, 16). Decrease in entrapment efficiency of all formulations at accelerated conditions can be ascribed to leakage of drug due to increase in water content.

Based on the results of the stability studies, RGD-grafted optimized Liposomal formulation was found to be stable under accelerated as well as long term storage condition. However, to ensure reduction in the moisture content relative to long term storage condition formulation should be stored in refrigerator as water content may change the physical stability on aging at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH).

## 6.4 References

1. Grit M, Crommelin DJ. The effect of aging on the physical stability of liposome dispersions. Chemistry and physics of lipids. 1992 Sep;62(2):113-22.
2. Cliff RO, Ligler F, Goins B, Hoffmann PM, Spielberg H, Rudolph AS. Liposome encapsulated hemoglobin: long-term storage stability and in vivo characterization. Biomaterials, artificial cells, and immobilization biotechnology.1992;20(2-4):619-26.
3. Fang JY, Lin HH, Hsu LR, Tsai YH. Characterization and stability of various liposome-encapsulated enoxacin formulations. Chemical & pharmaceutical bulletin. 1997 Sep;45(9):1504-9. PubMed PMID: 9332003. Epub 1997/10/23. eng.
4. Slabbert C, Plessis LH, Kotze AF. Evaluation of the physical properties and stability of two lipid drug delivery systems containing mefloquine. Int J Pharm. 2011 May 16;409(1-2):209-15.
5. Mowri H, Nojima S, Inoue K. Effect of lipid composition of liposomes on their sensitivity to peroxidation. Journal of biochemistry. 1984 Feb;95(2):551-8.
6. Grit M, Zuidam NJ, Underberg WJ, Crommelin DJ. Hydrolysis of partially saturated egg phosphatidylcholine in aqueous liposome dispersions and the effect of cholesterol incorporation on hydrolysis kinetics. J Pharm Pharmacology. 1993 Jun;45(6):490-5.
7. Frokjaer S, Hjorth EL, Worts O. Stability testing of liposomes during storage. In: Gregoriadis G, editor. Liposome Technology: Preparation of Liposomes. Boca Raton, Florida, US: CRC Press; 1984. p. 235-45.

8. Kasper JC, Schaffert D, Ogris M, Wagner E, Friess W. Development of a lyophilized plasmid/LPEI polyplex formulation with long-term stability—A step closer from promising technology to application. J Control Release. 2011 5/10/;151(3):246-55.

9. Cherng JY, Talsma H, Crommelin DJ, Hennink WE. Long term stability of poly((2-dimethylamino)ethyl methacrylate)-based gene delivery systems. Pharm. Res. 1999 Sep;16(9):1417-23.

10. Kasper JC, Troiber C, Kuchler S, Wagner E, Friess W. Formulation development of lyophilized, long-term stable siRNA/oligoaminoamide polyplexes. Eur J Pharm Biopharm. 2013 Oct;85(2):294-305.

11. Tang X, Pikal M. Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice. Pharm Res. 2004;21(2):191-200.

12. Shah SP, Misra A. Liposomal amikacin dry powder inhaler: effect of fines on in vitro performance. AAPS PharmSciTech. 2004;5(4):e65.

13. Abdelwahed W, Degobert G, Fessi H. Investigation of nanocapsules stabilization by amorphous excipients during freeze-drying and storage. Eur J of Pharm and Biopharm. 2006 Jun;63(2):87-94.

14. Chan HK, Clark AR, Feeley JC, Kuo MC, Lehrman SR, Pikal-Cleland K, et al. Physical stability of salmon calcitonin spray-dried powders for inhalation. J Pharm Sci. 2004 Mar;93(3):792-804.

15. Mattern M, Winter G, Kohnert U, Lee G. Formulation of proteins in vacuum-dried glasses. II. Process and storage stability in sugar-free amino acid systems. Pharm Dev. Tech. 1999 May;4(2):199-208.

16. Kasper JC, Schaffert D, Ogris M, Wagner E, Friess W. Development of a lyophilized plasmid/LPEI polyplex formulation with long-term stability--A step closer from promising technology to application. Journal of controlled release, 2011 May 10;151(3):246-55.

17. Claus S. Novel dry powder inhalation system based on dispersion of lyophilisates: Ludwig Maximilian University of Munich; 2012.