CHAPTER 9

STABILITY STUDY

9.1 Introduction

Stability study of formulation is carried out to provide evidence to answer the question of how the quality of the developed formulation changes with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a shelf life for the drug product and recommended storage conditions. The choice of test conditions defined in this guideline is based on an analysis of the effects of climatic conditions in the regions where the product is supposed to be marketed. The booming entry of any of the new dosage forms into market depends upon a well-defined stability study that can set up product's integrity, without doubt, at the end of study.

Stability protocol is divided into storage conditions and parameters to be looked into during storage. Hence, before starting stability program one must recognize key product characteristics, maintenance of which during stability will ensure desired performance of the product. Therefore, it is required to set up limits for each of the product parameters of interest prior to charging them for stability study.

Micelles suffer from environmental alteration upon administration such as dilution, pH and salt, and contact with numerous proteins and cells. Although the drug release from such micelles is a spontaneous process directed towards equilibrium, however; micelle as a drug cargo should release negligible amount of drug while stored in the container until used. The stability of micelles is explained in terms of thermodynamic and kinetic stability. Formation of micelle is explained by thermodynamic stability and its behavior over time, rate of polymer exchange and micelle disassembly is explained by Kinetic stability. Higher kinetic stability will ascertain that minimum amount of drug is leached outside during rearrangements. The stability of pH, dilution stability, particle size and zeta potential can be used as an indicator of thermodynamic and kinetic stability.

In case of colloidal drug delivery system size distribution and zeta potential are also important to reduce aggregation induced phase separation to yield long term stability. Micelle size of formulation also influences the entry of micelle system into the cells so these parameters are important to evaluate the efficiency of system. In present investigation, stability was accessed by determining percentage drug retained, pH of formulation zeta potential and micelle size of the formulations. These parameters have been evaluated initially

after optimization and during three months of storage at different temperature conditions. The stability protocol was designed as per in house protocol of our lab.

9.2 Method for Stability Study and Characterization Parameters

To test the storage stability of optimized batch of mixed micelle formulations containing Nicergoline were stored in triplicate in nasal spray bottles supplied by Micro Lab Mumbai, at the conditions as per in house protocol of our lab i.e. at refrigerated conditions (2-8ºC) and ambient room condition (25ºC±2ºC, for three months, the bottle was sealed with spray cap to minimize effect of humidity. The stability of the micelles was evaluated by withdrawing sample at regular interval of 30 days for a period of 90 days, and analyzed for size distribution, zeta potential, pH of formulation, dilution effect and drug leakage (1).

9.2.1 Dilution stability and Uniformity in dispersion

The 1ml of optimized micelle formulation was diluted to 50 ml using phosphate buffer pH 7.4. The dispersion was checked for any precipitation i.e leakage of drug from the micelle after dilution visually for 1 hr (2).

9.2.2 Assay and Drug retention

For assay 1 ml of micelle formulation was sonicated and dissolved in acetonitrile and after proper dilution analyzed using HPLC as described in analytical method development chapter. In case of drug retention the formulation was centrifuged at 5000 rpm for 10 min and supernatant as well as sediment was analyzed to study the amount of drug leakage on storage.

9.2.3 Micelle Size and Zeta Potential

The size of micelles was determined by laser diffraction using Particle Size Malvern Zetasizer Nano ZS (Zeta Sizer, Malvern, UK). Micelle formulations were diluted with distilled water. Formulation was filled in disposable polystyrene cuvette and analyzed for micelle size and zeta potential

9.2.4 pH of formulation

pH of formulation is important parameter as change in pH of formulation have an impact on nasal tissue. The dispersed formulation pH was measured using pH meter (pH meter, LabIndia, India).

9.2.5 % Transmittance

The % transmittance is direct indicator of clarity of formulation and was used as an indicator of any precipitation drug during storage period, which will result into decreased transmittance. The % transmittance of the micelle was checked against distilled water using UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan) at 630nm (**Table 9.1**).

9.2.6 Stirring

The formulation was stirred at 100 rpm for 48hrs and its stability was checked.

9.3 Results and Discussion

9.3.1 Dilution stability and Uniformity in dispersion

No precipitation of drug was observed during storage period even after dilution. But after 60 days of storage at RT precipitation was observed without dilution. Precipitation at RT is mainly due to leakage of drug from micelle structure.

Table 9.1 Dilution stability of micelle

Sampling Time	**Dilution stability**		**Transmittance (n=3) (Mean ± S.E.M)**	
	2-8ºC	RT	2-8ºC	RT
0 days	------	No precipitation	99.1 ± 2.6	99.1 ± 1.4
15 days	No precipitation	No precipitation	98.4 ± 2.5	97.3 ± 1.9
30 days	No precipitation	No precipitation	98.9 ± 2.6	91.1 ± 2.0
60 days	No precipitation	Precipitate	97.4 ± 1.9	89.8 ± 2.9
90 days	No precipitation	Precipitate	97.3 ± 1.6	87.6± 2.7

9.3.2 Assay, Drug retention, Size, pH, Size, Zeta Potential

Initial drug present in the formulation was considered to be 100% and the subsequent reduction was calculated as % of the initial drug remaining. The study was carried out for three months (3). The results are as shown in **Table 9.2**.

Table 9.2 Stability compilation of micelle formulation

Sampling Time (Days)	% Assay		% Drug retention		pH		Size (nm)		Zeta Potential	
	2-8ºC	RT	2-8ºC	RT	2-8ºC	RT	2-8ºC	RT	2-8ºC	RT
0	99.23 ± 1.63		99.23 ± 1.63		6.4 ± 0.13		16.53 ± 1.6		-14.3 ± 0.8	
15	98.11 ± 2.32	98.40 ± 1.83	99.23 ± 1.64	97.72 ± 2.66	6.4 ± 0.44	5.9 ± 0.74	17.54 ± 3.3	19.11 ± 2.2	-10.2 ± 0.5	- 9.3 ± 0.6
30	98.66 ± 1.69	98.27 ± 2.81	98.23 ± 2.23	93.50 ± 1.92	6.5 ± 0.62	6.1 ± 0.15	20.53 ± 2.8	22.53 ± 2.9	-5.6 ± 0.1	- 6.6 ± 0.9
60	98.25 ± 2.72	97.28 ± 1.69	97.57 ± 2.61	89.33 ± 2.32	6.4 ± 0.88	6.3 ± 0.17	21.84 ± 1.6	23.11 ± 1.6	-5.4 ± 1.0.	-5.7 ± 1.2
90	98.44 ± 2.51	97.33 ± 2.39	96.38 ± 1.83	83.29 ± 2.91	6.6 ± 0.42	5.7 ± 0.17	22.42 ± 3.6	25.88 ± 3.1	-5.3 ± 1.1	- 4.7 ± 1.6

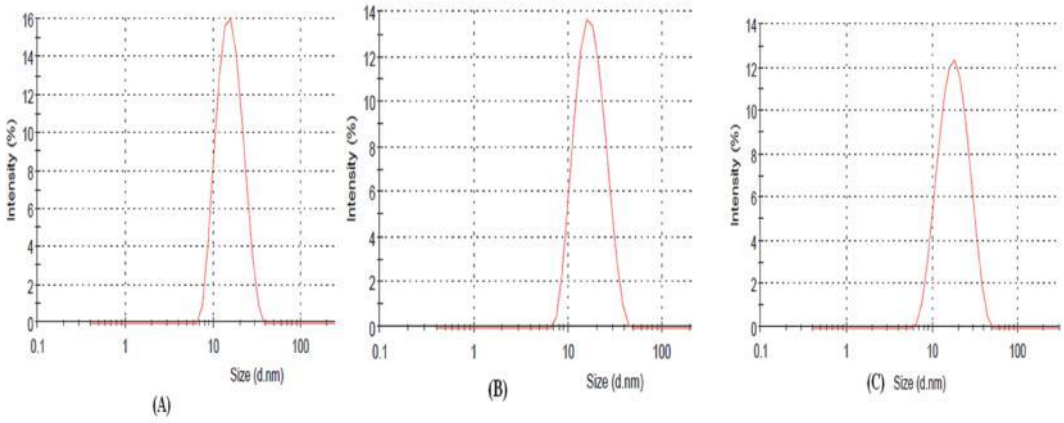

Figure 9.1 PF 127/TPGS micelle particle size at 25 °C and 100 rpm shaking (A: Before incubation; B: after 24 h incubation; C: after 48 h incubation).

Figure 9.1 presents the particle size of the Nicergoline-loaded mixed micelles during 48 h incubation at 25 °C with shaking at the speed of 100 rpm. No significant changes in micelle size or size distribution were recorded, which supports the high stability of Nicergoline-loaded PF-127/TPGS micelles.

9.4 Conclusion

The drug retention results of storage at 2-8ºC shows that data meets the requirements of "significant change" i.e. less than 5% change from its initial value. However, the storage at RT resulted in significant decrease in drug retention as a result of release of drug from micelle into the aqueous phase. From this we can say that at RT formulation is unable to hold the drug within the micelles, which, when comes into aqueous phase as a consequence of spontaneous equilibrium, gets precipitated due to its low water solubility. The higher stability at lower temperatures may be due to increased viscosity of the formulation which lowers the diffusion of the drug from micelles into solutions. So, suitable storage conditions for micellar formulations are at 2-8ºC. The analysis of micelle size during stability shows that there was an increase in size of about 5 nm on storage at 2-8ºC where as those stored at RTºC showed an increase in size of about 9 nm. Further, the zeta potential values were found to be decreased from -14 to -5.3 when stored at refrigerated conditions and to -4.7 when stored at RT. This shows that storage conditions had insignificant effect on the zeta potentials, and would be stable against aggregation at both conditions. When the micelle formulation stored at 2-8ºC was diluted with water, it was readily dispersed in water without any sign of precipitation at all sampling time points. However, formulations stored at RT showed precipitation at 60 and 90 days, due to significant release of water insoluble drug in the aqueous phase. The pH of the formulations was found to be maintained within the range of nasal cavity secretions and hence would not cause nasal irritation on application. Finally, it was concluded that micelle formulation was found to be stable for three months at refrigeration temperatures as no precipitation or aggregation was observed during storage.

9.5 References

1. Gao Y, Li LB, Zhai G. Preparation and characterization of Pluronic/TPGS mixed micelles for solubilization of camptothecin. Colloids and Surfaces B: Biointerfaces. 2008 7/15/;64(2):194-9.

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3. Dabholkar RD, Sawant RM, Mongayt DA, Devarajan PV, Torchilin VP. Polyethylene glycol–phosphatidylethanolamine conjugate (PEG–PE)-based mixed micelles: Some properties, loading with paclitaxel, and modulation of P-glycoprotein-mediated efflux. Int J Pharm. 2006 6/6/;315(1–2):148-57.