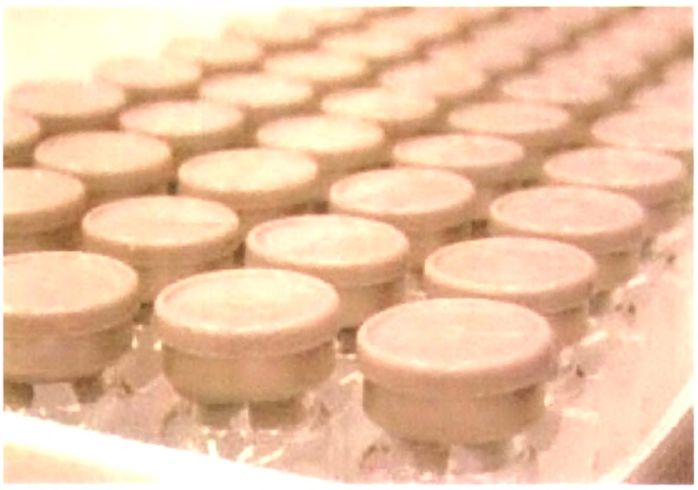
Chapter 7

Stability Studies



7.1. Introduction

Stability is defined as the capacity of a drug substance or drug product to remain within established specifications to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (Draft guidance, Stability Testing of Drug Substances and Drug Products, FDA, 1998). The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and light, and to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions (Draft guidance, Stability Testing of New Drug Substances and Products, 2003). Physical, chemical, and microbiological data are generated as a function of time and storage conditions (e.g., temperature and relative humidity [RH]). It is a well-known fact that for drug delivery systems, stability of the formulation is one of the most critical parameters from the pharmaceutical aspect. The storage conditions are particularly important to define in order to start biological studies and to make sure that the drug doses used would be preserved. For this purpose, accelerated stability testing at high temperatures and humidity conditions are often employed to predict the shelf life of drugs.

Particulate delivery systems like microparticles and nanoparticles are widely used to deliver a wide range of drugs. The nanoparticles protect the drug from metabolizing enzymes, sustain the release to be administered orally or injected locally and target specific tissues by incorporating surface ligand moieties. Chitosan-based nanoparticles loaded or unloaded and of positive zeta potential, showed instability towards physiological salt solutions and physiological pH values. Aggregation phenomena were found to be time and temperature dependent (Käuper et al., 2007). Although, the instability of the nanoparticles in the dispersion is overcome by lyophilization using cryoprotectants. The influence of the storage conditions like temperature and humidity on the particle size and drug content are important in maintaining the integrity of these delivery systems before animal studies (Kotze et al., 1999, Agnihotri et al., 2004).

7.2. Methodology

The stability studies were carried out in accordance with the ICH guidelines for new drug products. The stability studies were carried out for the nanoparticles formulations at 5°C ± 3°C for 6 months and 25°C ± 2°C/60 ± 5 % RH up to 6 months.

Three optimized stability batches were prepared and subjected to stability studies. The nanoparticles were filled in glass vials, closed with rubber closures and sealed with aluminum caps. The samples were withdrawn at predetermined levels and were examined visually for physical appearance. The contents of the vials were evaluated for the particle size, zeta potential and drug content.

Statistical Analysis and Data Interpretation

Data of stability experiments were expressed as Mean ± SD. The data were compared using ANOVA and student's t-test and difference larger than the value at $p<0.05$ were considered significant.

"Significant change" *was* considered under following conditions

- A 5 percent change in assay from its initial value
- Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test, particle size and drug content may be expected under accelerated conditions.

The results of stability studies are recorded in Tables 7.1 to Table 7.6 and shown in Figures 7.1 to Figure 7.6.

7.3. Results and Discussions

The stability studies of the formulations were performed in order to study the influence of varying environmental conditions on formulation parameters influencing the therapeutic response. The stability studies were carried out in accordance with the ICH guidelines for new drug substances or product intended to be stored in a refrigerator. The stability of the nanoparticles were assessed for physical observation, particle size, zeta potential and the drug content (with respect to the initial) at 5°C ± 3°C for 6M and 25°C ± 2°C/60% RH ± 5% RH for 6M. The drug content in the initial sample was considered as 100 percent. For accelerated condition (i.e. 25°C ± 2°C/60% ± 5 %RH) the sampling was done at 1, 2, 3, 6 months and for 5°C ± 3°C the sampling was done at 1, 3, 6 months.

The results of stability studies are recorded in Tables 7.1 to Table 7.6 and shown in Figures 7.1 to Figure 7.6.

Table: 7.1. Stability studies data of LMC-TZ NPs & LMC-CBZ NPs

Stability conditions	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content (%)	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content %
	LMC-TZ NPs				LMC-CBZ NPs			
Initial	Free flowing white powder with easy redispersibility	473.5± 13.5	22.8±0.87	100.0 ± 3.5*	Free flowing white powder with easy redispersibility	472.6±12.7	24.7±0.98	100.0 ±6.1*
5°C ± 3°C								
1 M	Free flowing white powder with easy redispersibility	479.4 ± 13.8	23.1 ± 1.5	98.9 ± 2.4	Free flowing white powder with easy redispersibility	478.8 ± 11.5	24.1 ± 1.2	98.4 ± 2.8
3 M	Free flowing white powder with easy redispersibility	489.7 ± 13.8	23.4 ± 1.7	97.7 ± 2.5	Free flowing white powder with easy redispersibility	481.6 ± 14.8	23.9 ± 2.2	97.5 ± 1.9
6 M	Free flowing white powder with easy redispersibility	486.4 ± 12.9	22.9 ± 2.2	96.9± 1.8	Free flowing white powder with easy redispersibility	484.5 ±12.9	24.9 ±1.8	98.2± 2.1
25°C ± 2°C/60% RH ± 5% RH								
1 M	Free flowing white powder with easy redispersibility	478.3 ± 14.6	24.1 ± 2.7	98.9± 2.1	Free flowing white powder with easy redispersibility	479.9 ± 13.7	24.9 ± 2.6	99.1± 3.1
2 M	Free flowing white powder with easy redispersibility	491.4 ± 16.5	24.9 ± 3.1	97.9± 2.3	Free flowing white powder with easy redispersibility	496.8 ± 15.9	25.1 ± 3.9	97.2± 3.3
3 M	White powder with poor flow and difficult redispersibility	732.8 ± 19.6	19.8 ± 2.7	93.6± 2.7	Free flowing white powder with easy redispersibility	782.9 ± 17.4	17.7 ± 2.8	91.8± 1.7
6 M	White powder with poor flow and poor redispersibility	945.6 ± 20.7	15.6 ± 1.5	87.1 ± 2.5	Free flowing white powder with easy redispersibility	915.8 ± 19.7	14.8 ± 1.9	85.1 ± 2.9

* Initial drug content was labeled as 100% and the drug content at different time points are with respect to the initial drug content

Table: 7.2. Stability studies data of MMC-TZ NPs & MMC-CBZ NPs

Stability conditions	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content (%)	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content %
	MMC-TZ NPs				MMC-CBZ NPs			
Initial	Free flowing white powder with easy redispersibility	598.7±18	23.8 ± 0.98	100.0 ± 2.4*	Free flowing white powder with easy redispersibility	628.2±13.1	25.1±1.1	100.0 ± 6.4*
5°C ± 3°C								
1 M	Free flowing white powder with easy redispersibility	599.3 ± 12.8	23.1 ± 1.9	98.1 ± 2.9	Free flowing white powder with easy redispersibility	631.9 ± 18.5	25.9 ± 1.2	99.2 ±3.1
3 M	Free flowing white powder with easy redispersibility	597.9 ± 16.8	23.6 ± 1.3	96.8 ± 2.8	Free flowing white powder with easy redispersibility	633.1 ± 15.8	25.8 ± 2.5	97.1 ±3.1
6 M	Free flowing white powder with easy redispersibility	606.1 ± 18.9	24.9 ± 2.8	96.1± 1.1	Free flowing white powder with easy redispersibility	634.1 ±1 7.9	26.4 ±1.8	96.2± 1.9
25°C ± 2°C/60% RH ± 5% RH								
1 M	Free flowing white powder with easy redispersibility	598.9 ± 16.8	24.7 ± 1.5	98.5 ± 2.4	Free flowing white powder with easy redispersibility	632.8 ± 17.4	25.7 ± 1.6	98.5 ±2.1
2 M	Free flowing white powder with easy redispersibility	609.6 ± 19.8	24.9 ± 1.6	97.2 ± 2.9	Free flowing white powder with easy redispersibility	638.6± 19.3	25.9± 2.1	96.4 ±1.9
3 M	White powder with poor flow and difficult redispersibility	799.9 ± 25.7	17.9±3.8	94.8± 1.9	Free flowing white powder with easy redispersibility	884.9 ±14.9	18.5 ±1.9	93.2± 3.1
6 M	White powder with poor flow and poor redispersibility	965.9 ± 22.5	14.8 ± 2.5	88.2 ± 4.5	Free flowing white powder with easy redispersibility	995.5 ± 20.7	15.1 ± 1.2	86.2 ± 2.3

* Initial drug content was labeled as 100% and the drug content at different time points are with respect to the initial drug content

Table: 7.3. Stability studies data of LMTC-TZ NPs & LMTC-CBZ NPs

Stability conditions	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content (%)	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content %
	LMTC-TZ NPs				LMTC-CBZ NPs			
Initial	Free flowing white powder with easy redispersibility	262.5 ±12.4	12.9±1.44	100.0 ± 6.3*	Free flowing white powder with easy redispersibility	272.1±11.5	20.9±1.7	100.0 ± 4.3
5°C ± 3°C								
1 M	Free flowing white powder with easy redispersibility	269.7 ± 16.3	13.4 ± 1.7	98.7 ± 2.3	Free flowing white powder with easy redispersibility	271.7 ± 13.5	21.2± 1.7	98.7 ±1.7
3 M	Free flowing white powder with easy redispersibility	267.3 ± 15.7	13.7 ± 1.7	97.7 ± 2.9	Free flowing white powder with easy redispersibility	278.8 ± 12.8	21.8 ± 1.3	98.1 ±2.1
6 M	Free flowing white powder with easy redispersibility	268.9 ± 19.2	13.9 ± 2.5	97.1± 1.7	Free flowing white powder with easy redispersibility	274.7 ±1 4.7	21.6±1.2	97.9± 1.8
25°C ± 2°C/60% RH ± 5% RH								
1 M	Free flowing white powder with easy redispersibility	265.8 ± 17.8	13.1 ± 1.4	98.7 ± 2.3	Free flowing white powder with easy redispersibility	279.1 ± 14.2	21.7 ± 1.3	98.9 ±3.1
2 M	Free flowing white powder with easy redispersibility	271.5 ± 12.7	14.2 ± 1.6	97.7 ± 2.9	Free flowing white powder with easy redispersibility	281.8± 13.7	21.9 ± 1.1	97.7 ±1.8
3 M	White powder with poor flow and difficult redispersibility	499.8 ± 22.7	10.07± 1.8	95.4± 1.7	Free flowing white powder with easy redispersibility	504.6 ±17.7	16.9±1.7	94.1± 2.1
6 M	White powder with poor flow and poor redispersibility	665.1 ± 23.6	9.2 ± 0.59	87.9 ± 2.3	Free flowing white powder with easy redispersibility	699.3 ± 23.9	14.9 ± 1.1	89.9 ± 2.4

* Initial drug content was labeled as 100% and the drug content at different time points are with respect to the initial drug content

Table: 7.4. Stability studies data of MMTC-TZ NPs &MMTC-CBZ NPs

Stability conditions	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content (%)	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content %
	MMTC-TZ NPs				MMTC-CBZ NPs			
Initial	Free flowing white powder with easy redispersibility	264.9 ±15	14.5 ± 1.1	100.0 ± 3.4*	Free flowing white powder with easy redispersibility	282.9±15.6	27.7±1.2	100.0 ± 3.2*
5°C ± 3°C								
1 M	Free flowing white powder with easy redispersibility	268.2 ± 13.5	14.4 ± 1.6	98.9 ± 2.5	Free flowing white powder with easy redispersibility	281.8 ± 16.2	26.9± 1.4	98.5 ±1.4
3 M	Free flowing white powder with easy redispersibility	269.2 ± 13.7	14.8 ± 1.6	98.6 ± 2.4	Free flowing white powder with easy redispersibility	288.1 ± 17.8	27.9 ± 1.2	98.8 ±1.9
6 M	Free flowing white powder with easy redispersibility	268.3 ± 12.1	14.9 ± 2.7	98.1± 1.6	Free flowing white powder with easy redispersibility	284.5 ±1 3.8	28.1 ±1.5	98.3± 1.4
25°C ± 2°C/60% RH ± 5% RH								
1 M	Free flowing white powder with easy redispersibility	267.5 ± 11.8	14.7 ± 1.4	99.1 ± 2.4	Free flowing white powder with easy redispersibility	284.7 ± 17.2	27.7 ± 1.6	98.6 ±2.1
2 M	Free flowing white powder with easy redispersibility	272.2 ± 13.8	14.9 ± 1.3	97.8 ± 2.3	Free flowing white powder with easy redispersibility	285.9± 16.7	28.3 ± 1.8	97.6 ±1.4
3 M	White powder with poor flow and difficult redispersibility	519.9 ± 12.9	11.6± 1.2	94.7± 1.9	Free flowing white powder with easy redispersibility	608.6 ±15.4	18.8 ±1.9	93.1 2.8
6 M	White powder with poor flow and poor redispersibility	675.8 ± 13.3	9.8 ± 0.89	89.4 ± 3.5	Free flowing white powder with easy redispersibility	677.3 ± 14.8	15.1 ± 1.8	88.3 ± 2.7

* Initial drug content was labeled as 100% and the drug content at different time points are with respect to the initial drug content

Table: 7.5. Stability studies data of LMTMC-TZ NPs & LMTMC-CBZ NPs

Stability conditions	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content (%)	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content %
	LMTMC-TZ NPs				LMTMC-CBZ NPs			
Initial	Free flowing white powder with easy redispersibility	168.0±10.2	13.4 ± 1.1	100.0 ± 2.2*	Free flowing white powder with easy redispersibility	184.6±13.9	13.9±1.2	100.0 ± 5.4*
5°C ± 3°C								
1 M	Free flowing white powder with easy redispersibility	169.9 ± 12.5	13.6 ± 1.4	98.8 ± 2.3	Free flowing white powder with easy redispersibility	185.7 ± 14.5	13.8± 1.3	98.9 ±1.5
3 M	Free flowing white powder with easy redispersibility	171.8 ± 16.7	13.8 ± 1.7	98.1 ± 2.3	Free flowing white powder with easy redispersibility	188.6 ± 13.9	14.1 ± 1.1	98.1 ±1.8
6 M	Free flowing white powder with easy redispersibility	170.8 ± 13.5	14.2 ± 2.1	97.9± 1.9	Free flowing white powder with easy redispersibility	186.9 ±1 7.6	14.2 ±1.3	97.7± 1.3
25°C ± 2°C/60% RH ± 5% RH								
1 M	Free flowing white powder with easy redispersibility	167.5 ± 15.7	13.7 ± 1.4	98.1 ± 2.6	Free flowing white powder with easy redispersibility	185.8 ± 13.9	13.7 ± 1.9	98.3±2.8
2 M	Free flowing white powder with easy redispersibility	172.8 ± 19.4	13.9 ± 1.9	98.8 ± 2.3	Free flowing white powder with easy redispersibility	189.5± 13.7	14.6 ± 1.2	97.5 ±2.1
3 M	White powder with poor flow and difficult redispersibility	319.7 ± 15.7	11.3± 1.1	93.9± 1.5	Free flowing white powder with easy redispersibility	408.9 ±18.4	11.8 ±0.84	94.6± 2.3
6 M	White powder with poor flow and poor redispersibility	499.5 ± 12.8	8.8 ± 0.83	88.6 ± 3.8	Free flowing white powder with easy redispersibility	547.1 ± 14.7	9.1 ± 0.98	89.4 ± 2.1

* Initial drug content was labeled as 100% and the drug content at different time points are with respect to the initial drug content

Table: 7.6. Stability studies data of MMTMC-TZ NPs & MMTMC-CBZ NPs

Stability conditions	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content (%)	Description & Redispersibility	Particle size (nm)	Zeta potential (mV)	Drug content %
	MMTMC-TZ NPs				MMTMC-CBZ NPs			
Initial	Free flowing white powder with easy redispersibility	226.6±18.1	16.7± 1.5	100.0 ± 1.8*	Free flowing white powder with easy redispersibility	274.5±21.3	15.9±1.1	100.0 ± 2.1*
5°C ± 3°C								
1 M	Free flowing white powder with easy redispersibility	229.6 ± 15.3	16.6 ± 1.2	98.7 ± 2.8	Free flowing white powder with easy redispersibility	278.5 ± 14.5	15.8± 1.5	98.3 ±1.2
3 M	Free flowing white powder with easy redispersibility	228.4 ± 13.3	16.9 ± 1.4	97.9 ± 2.1	Free flowing white powder with easy redispersibility	277.8 ± 15.8	16.1 ± 1.3	97.4 ±1.5
6 M	Free flowing white powder with easy redispersibility	230.8 ± 18.2	17.2 ± 2.1	96.5± 1.3	Free flowing white powder with easy redispersibility	280.1 ± 16.9	16.4 ±1.1	96.8± 1.4
25°C ± 2°C/60% RH ± 5% RH								
1 M	Free flowing white powder with easy redispersibility	227.6 ± 12.8	16.7 ± 1.8	98.4 ± 2.7	Free flowing white powder with easy redispersibility	275.3± 19.7	15.7 ± 1.4	98.9±2.1
2 M	Free flowing white powder with easy redispersibility	232.6 ± 16.5	16.9 ± 1.4	97.3 ± 2.1	Free flowing white powder with easy redispersibility	281.3± 18.4	16.6 ± 1.5	97.7 ±1.8
3 M	White powder with poor flow and difficult redispersibility	419.4 ± 17.6	12.4± 1.4	92.9± 1.4	Free flowing white powder with easy redispersibility	469.3 ±18.1	13.1 ±1.1	93.5± 2.9
6 M	White powder with poor flow and poor redispersibility	589.7 ± 19.8	9.5 ± 0.78	90.1 ± 3.2	Free flowing white powder with easy redispersibility	579.5 ± 19.3	10.5 ± 0.97	87.7 ± 2.6

* Initial drug content was labeled as 100% and the drug content at different time points are with respect to the initial drug content

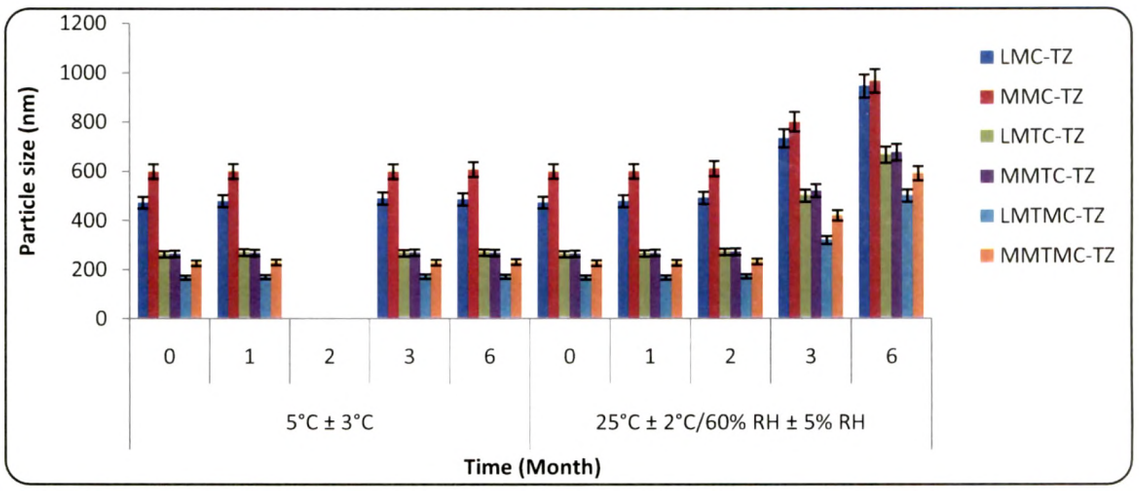


Figure 7.1: Stability profiles of tizanidine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs on particle size

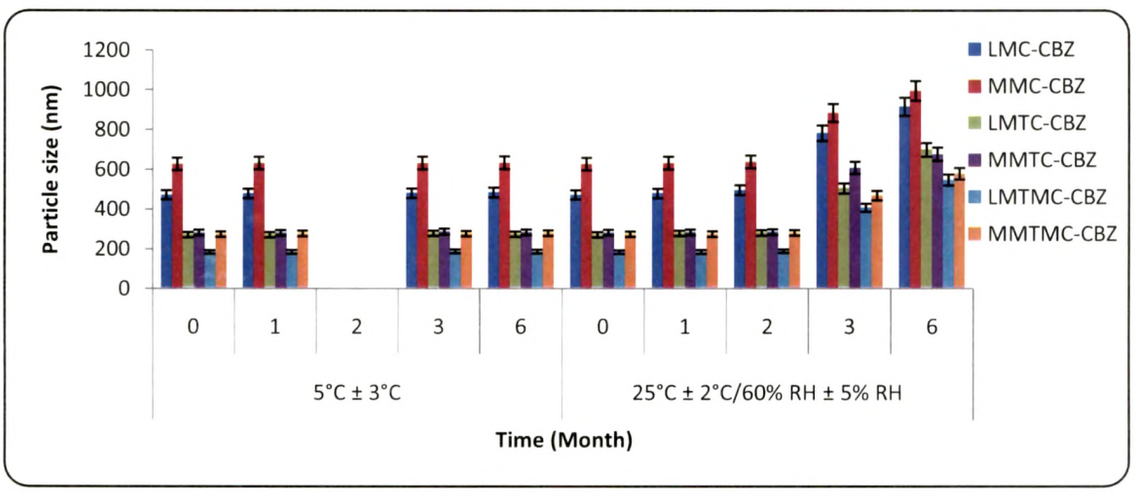


Figure 7.2: Stability profiles of cyclobenzaprine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs on particle size

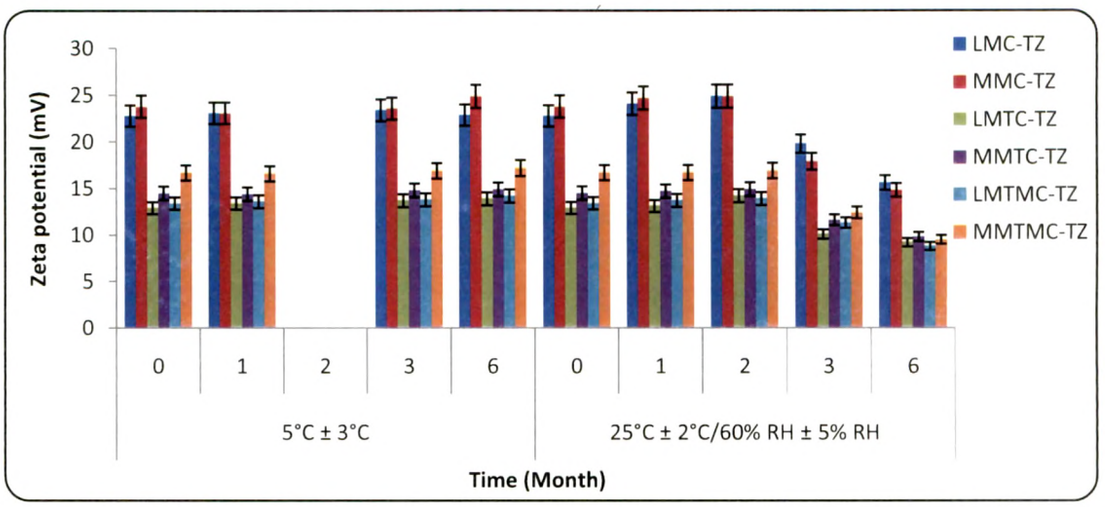


Figure 7.3: **Stability profiles of tizanidine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs on Zeta potential**

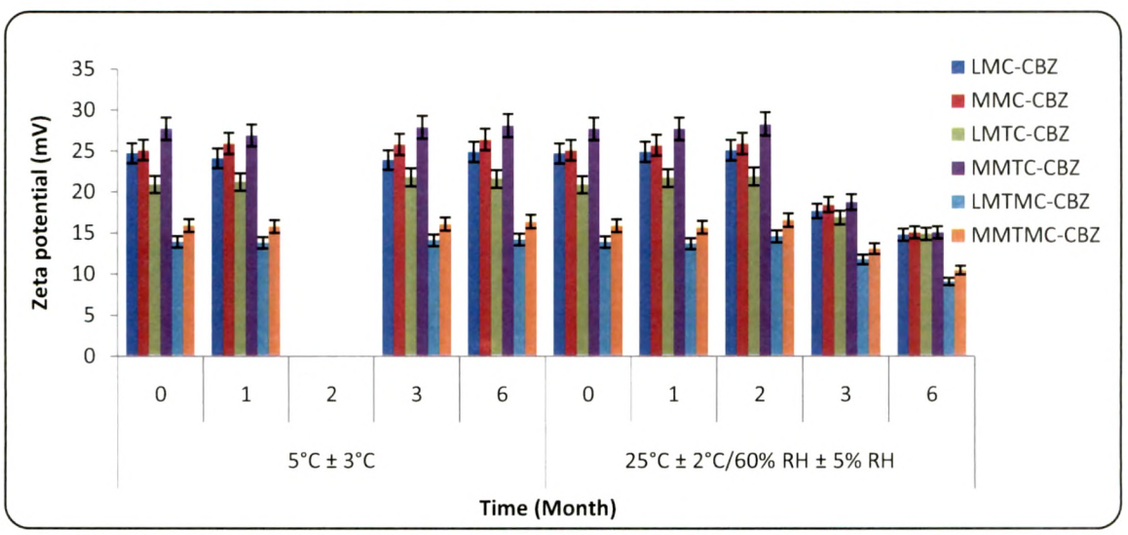


Figure 7.4: **Stability profiles of cyclobenzaprine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs on Zeta potential.**

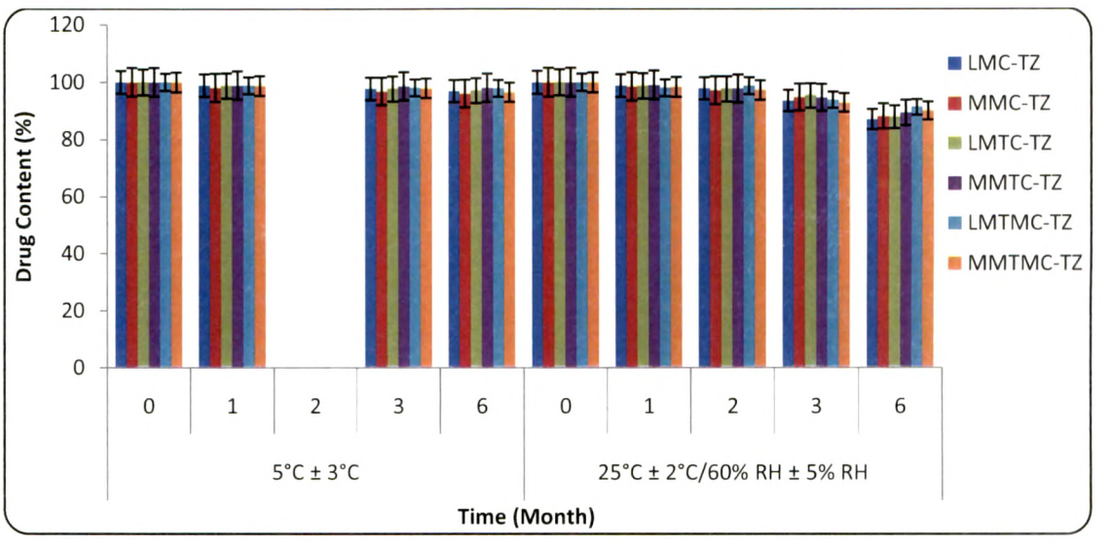


Figure 7.5: Stability profiles of tizanidine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs on Drug content

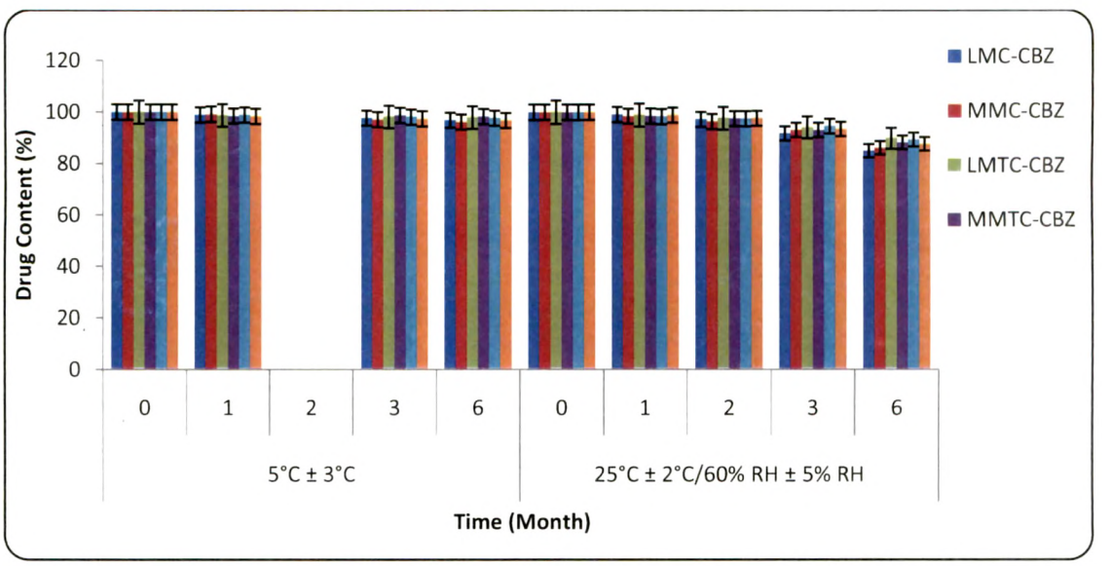


Figure 7.6: Stability profiles of cyclobenzaprine HCl loaded chitosan, thiolated chitosan and trimethyl chitosan NPs on Drug content

It was observed that TZ loaded and CBZ loaded chitosan, thiolated chitosan and trimethyl chitosan NPs have no significant change ($P>0.05$) observed in particle size, zeta potential and drug content at 5°C ± 3°C for 6M. The storage of the TZ loaded and CBZ loaded chitosan, thiolated chitosan and trimethyl chitosan NPs at 25°C ± 2°C/60% ± 5% RH, led to increase in the particle size. The increase in the particle size was not significant during the first month, however became significant and more prominent after 2, 3 and 6 months. During our analysis of samples, the polydispersity

index of the nanoparticles stored at 25°C ± 2°C/60% ± 5% RH was found to increase as compared to the initial. The increased in the particle size may be due to the absorption of the moisture by the nanoparticles resulting in the coalescence of the small nanoparticles forming particles larger in size.

The nanoparticles were also observed for physical appearance. After 3 and 6 months the physical appearance was also changed, with loss of the free flowing property followed by the difficulty in redispersibility. Also, the thiolated chitosan nanoparticles demonstrated difference in the color than the initial powder. At 6 months the color of the powder was yellow. This could be indicative of the oxidation of thiol group of the surface.

At 25°C ± 2°C/60% ± 5% RH, the zeta potential of the nanoparticles shifted towards the zero for TZ loaded and CBZ loaded chitosan, thiolated chitosan and trimethyl chitosan NPs. The lowered zeta potential values also might have contributed toward the aggregation of particles.

The initial drug entrapment of LMC-TZ NPs, LMC-CBZ NPS, MMC-TZ NPs, MMC-CBZ NPs, LMTC-TZ NPs, LMTC-CBZ NPs, MMTC-TZ NPs, MMTC-CBZ NPS, LMTMC-TZ NPs, LMTMC-CBZ NPs, MMTMC-TZ NPs and MMTMC-CBZ NPs was found to be 44.69±3.5, 62.27±6.1, 46.65±2.4, 64.14±6.4, 68.8±6.3, 70.45±4.3, 72.2±3.4, 80.20±3.2, 66.90±1.2, 71.75±5.4, 62.27±1.8 and 72.88±2.1 respectively. The drug content of the TZ loaded and CBZ loaded chitosan, thiolated chitosan and trimethyl chitosan NPs nanoparticles was not altered up to 6M at 5°C ± 3°C. However, the drug content was reduced after 6M storage at 25°C ± 2°C/60% RH ± 5% RH. This impact could be due to the moisture absorbed by the nanoparticles upon storage at 25°C ± 2°C/60% RH ± 5% RH, possibly resulting in the degradation of the drug.

The release profile of the drug from the nanoparticles was not affected upon storage. The similarity factor calculated for the between the initial and the 6M samples show values greater than 80, indicating high similarity between the initial and 6M.

7.4. Conclusions

From the above study, we may concluded that the TZ loaded and CBZ loaded chitosan, thiolated chitosan and trimethyl chitosan NPs, when stored at 25°C ± 2°C/60% RH ± 5% RH for 6M show instability reflected by change in physical appearance, increase in the particle size, zeta potential and reduction in the drug content. Hence, we can conclusively specify that both TZ loaded and CBZ loaded

chitosan, thiolated chitosan and trimethyl chitosan NPs were stable and can be stored 5°C ± 3°C for 6M retaining its original formulation characteristics. Further, long term stability should be carried out to assess the influence of stability parameters on the stability of the prepared NPs at 5°C ± 3°C.

7.5. References

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