CHAPTER 6

STABILITY STUDIES

6. STABILITY STUDIES

6.1 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to maintain its identity, strength, quality, and purity throughout the retest or expiration dating periods (Draft guidance, Stability Testing of Drug Substances and Drug Products, FDA, 1998).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a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions (Draft guidance, Stability Testing of New Drug Substances and Products, 2003).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, be administered orally or injected locally, and target specific tissues by incorporating surface ligand moieties. Poly (lactide), poly(glycolide) and their copolymers approved by the U.S. Food and Drug Administration (FDA) represent a major class of synthetic biodegradable materials essentially useful for the preparation of microparticles and nanospheres. The factors that influence the chemical degradation of PLGA are well known and include polymer molecular weight, ratio of lactic to glycolic acid in the co-polymers, polymer-drug ratio, environmental temperature, pH, and geometry of the delivery system (Burcu Sayin and Sema Calis, 2004;Gasper MM

et al, 1998). The main mode of degradation for the PLGA polymer is purely through simple hydrolysis of the ester bonds and does not involve any enzymatic activity.(Mauduit J 1996) In vivo it degrades into lactic acid and glycolic acid. Lactic acid enters the tricarboxylic acid cycle and is metabolized and subsequently eliminated from the body as carbon dioxide and water. Glycolic acid is either excreted unchanged in the kidney or it enters the tricarboxylic acid cycle and is eventually eliminated as carbon dioxide and water. (Burcu Sayin and Sema Calis, 2004) It has been shown that PLGA nanospheres and microspheres have a shelf-life of more than 3 months (PLGA 50:50, 0.63 dL/g) (Feng S, 2001).

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 use for the biological studies.

6.2 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 nanoparticle formulations at 5°C ± 3°C for 6 months and (25°C ± 2°C/60 ± 5 % RH) up to 6 months. Three batches at optimized process and formulation conditions 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

Three batches of each formulation was evaluated three times, data of nine experiments are 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 6.1- 6.4 and figures 6.1-6.6.

6.3 Results and Discussion

The stability studies of the formulations were performed in order to study the influence of varying environmental conditions on the parameters of the formulation influencing the therapeutic response. The stability studies were carried out in accordance with the ICH guidelines for drug substances intended to be stored in a refrigerator. The stability of the nanoparticles was 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 6 months. 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, 6months.

The results for the stability studies are represented in table 6.1 and 6.2 for PLGA-ETP-NP and Tf-PLGA-ETP-NP respectively. The graphical representations are given in figure 6.1 and 6.2. The results for PLGA-TMZ-NP and Tf-PLGA-TMZ –NP are recorded in table 6.3 and 6.4 respectively. The results are graphically shown in figure 6.3 and 6.6.

Table: 6.1 Stability studies data of PLGA-ETP-NP

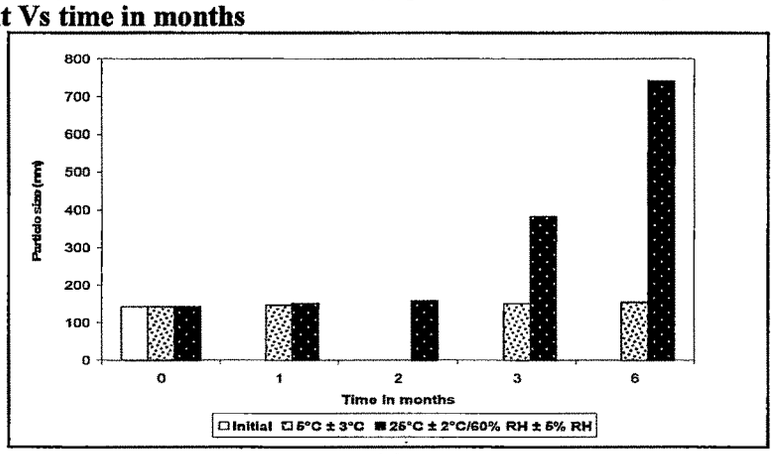
Stability conditions	**Description & Redispersibility**	**Particle size (nm)**	**Zeta potential (mV)**	**Drug content %**
Initial	Free flowing white powder with easy redispersibility	149 ± 8	-9.8 ± 1.3	100.0 ± 2.4*
5°C ± 3°C				
1 M	Free flowing white powder with easy redispersibility	152 ± 3	-8.9 ± 1.4	98.1 ± 2.5
3 M	Free flowing white powder with easy redispersibility	151 ± 6	-9.2 ± 1.5	97.9 ± 2.1
6 M	Free flowing white powder with easy redispersibility	150 ± 4	-8.1 ± 1.5	97.1± 1.9
25°C ± 2°C/60% RH ± 5% RH				
1 M	Free flowing white powder with easy redispersibility	152 ± 4	-10.4 ± 1.2	98.7± 2.0
2 M	Free flowing white powder with easy redispersibility	159 ± 8	-9.2 ± 1.4	97.2± 2.3
3 M	White powder with poor flow and difficult redispersibility	383 ± 9	-6.8 ± 1.0	96.8± 1.8
6 M	White powder with poor flow and poor redispersibility	743 ± 6	-3.6 ± 1.2	95.7 ± 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

Table: 6.2 Stability data of Tf-PLGA-ETP-NP

Stability conditions	**Description**	**Particle size (nm)**	**Zeta potential (mV)**	**Drug content %**
Initial	Free flowing white powder with easy redispersibility	162.1 ± 12	-11.69 ± 0.8	100.0± 2.5
5°C ± 3°C				
1 M	Free flowing white powder with easy redispersibility	165 ± 5	-10.9 ± 1.5	99.6 ± 2.5
3 M	Free flowing white powder with easy redispersibility	161 ± 7	-11.2 ± 1.2	99.8± 2.3
6 M	Free flowing white powder with easy redispersibility	168 ± 9	-11.6 ± 2.0	97.8± 1.9
25°C ± 2°C/60% RH ± 5% RH				
1 M	Free flowing white powder with easy redispersibility	167 ± 6	-10.6 ± 1.1	98.7± 2.0
2 M	Free flowing white powder with easy redispersibility	171 ± 5	-10.1 ± 1.2	97.8± 1.8
3 M	White powder with poor flow and difficult redispersibility	429 ± 11	-8.7 ± 2.1	96.7± 2.1
6 M	Light pink powder with poor flow and poor redispersibility	770 ± 13	-4.8 ± 1.6	97.1 ± 2.5

Fig 6.1 Stability profiles-PLGA-ETP-NP (a) particle size (b) zeta potential and (c) drug content Vs time in months



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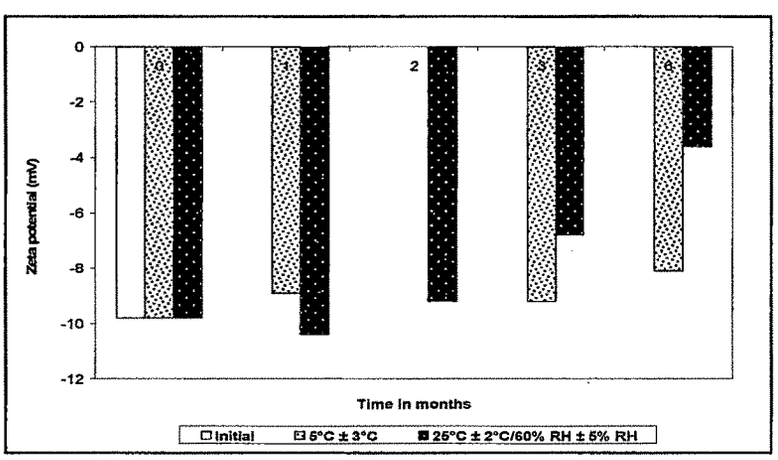

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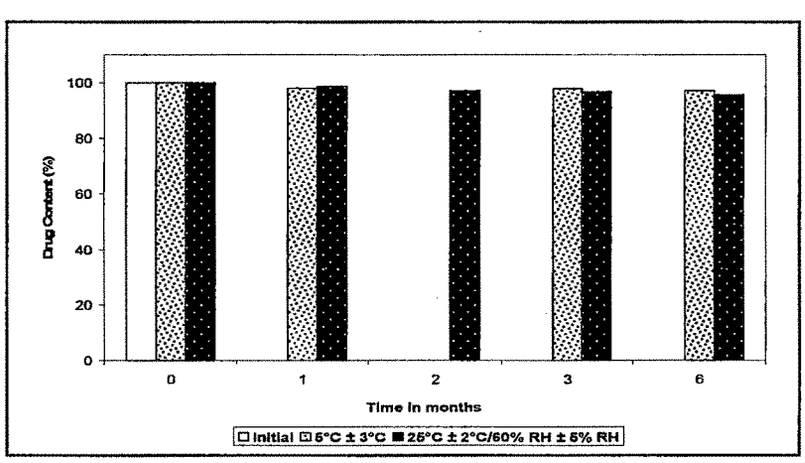

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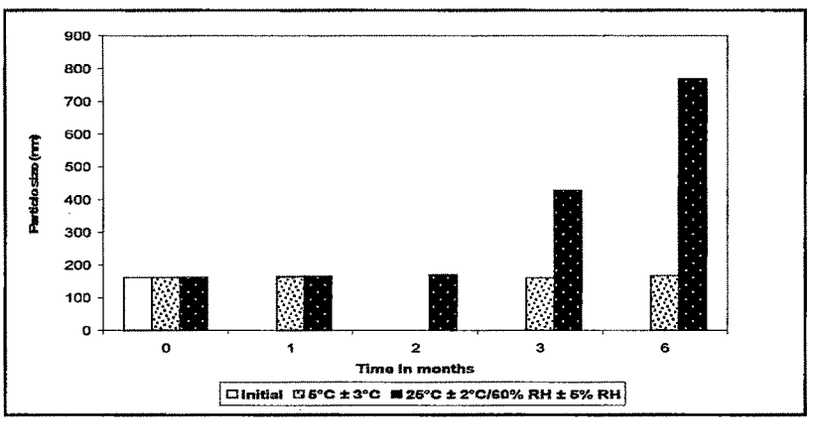
Fig 6.2 Stability profiles-Tf-PLGA-ETP-NP (a) particle size (b) zeta potential and (c) drug content Vs time in months



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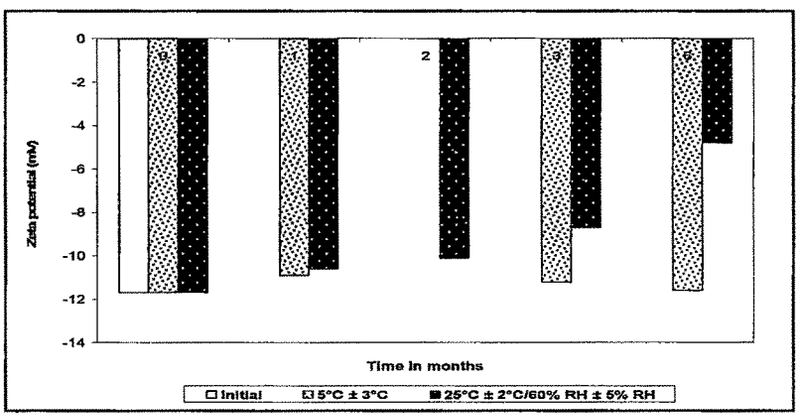
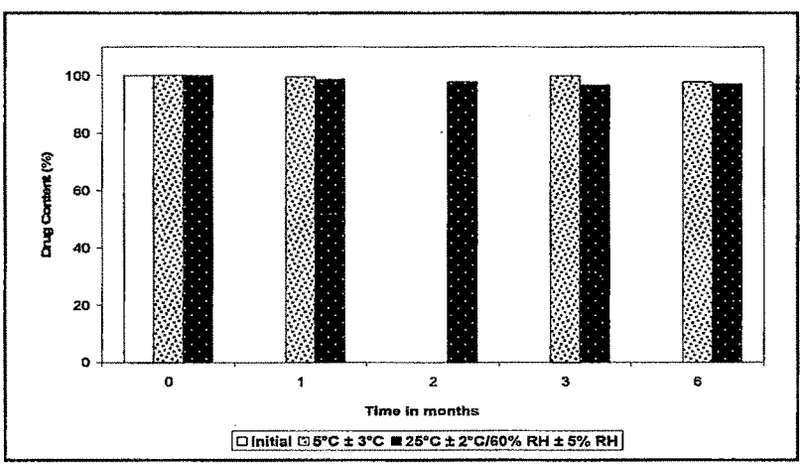


(b)



(c)

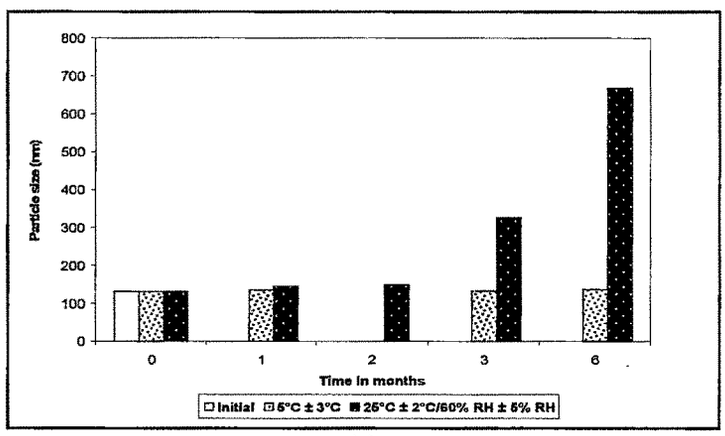
Table: 6.3 Stability data of PLGA-TMZ-NP

Stability conditions	**Description**	**Particle size (nm)**	**Zeta potential (mV)**	**Drug content %**
Initial	Free flowing white powder with easy redispersibility	138 ± 7	-7.8 ± 1.7	100.0± 2.9
5°C ± 3°C				
1 M	Free flowing white powder with easy redispersibility	136 ± 5	-8.2 ± 2.0	99.5± 1.3
3 M	Free flowing white powder with easy redispersibility	141 ± 6	-8.6 ± 1.5	98.7 ± 2.5
6 M	Free flowing white powder with easy redispersibility	143 ± 4	-7.4 ± 1.0	99.2± 1.9
25°C ± 2°C/60% RH ± 5% RH				
1 M	Free flowing white powder with easy redispersibility	136 ± 6	-9.1 ± 1.5	97.1 ± 2.1
2 M	Free flowing white powder with easy redispersibility	150 ± 5	-6.8 ± 0.5	96.4 ± 1.8
3 M	White powder with poor flow and difficult redispersibility	327 ± 8	-6.1 ± 1.0	94.3± 2.1
6 M	White powder with poor flow and poor redispersibility	669 ± 11	-3.8 ± 1.3	90.1 ± 1.3

Table: 6.4 Stability data of Tf-PLGA-TMZ-NP

Stability conditions	**Description**	**Particle size (nm)**	**Zeta potential (mV)**	**Drug content %**
Initial	Free flowing white powder with easy redispersibility	153.8 ± 14	-13.6 ± 3.1	100.0± 2.2
5°C ± 3°C				
1 M	Free flowing white powder with easy redispersibility	157 ± 6	-11.2 ± 3.2	98.0 ± 2.1
3 M	Free flowing white powder with easy redispersibility	152 ± 4	-13.1 ± 1.9	97.6 ± 1.7
6 M	Free flowing white powder with easy redispersibility	161 ± 5	-10.3 ± 2.5	98.7 ± 2.1
25°C ± 2°C/60% RH ± 5% RH				
1 M	Free flowing white powder with easy redispersibility	159 ± 9	-10.2 ± 1.2	98.1 ± 1.4
2 M	Free flowing white powder with easy redispersibility	163 ± 7	-9.1 ± 0.8	97.3 ± 2.1
3 M	White powder with poor flow and difficult redispersibility	390 ± 11	-7.3 ± 2.5	93.7 ± 1.9
6 M	Light pink powder with poor flow and poor redispersibility	688 ± 14	-4.6 ± 1.2	90.6 ± 2.3

Figure 6.3 Stability profiles- PLGA-TMZ-NP (a) particle size (b) zeta potential and (c) drug content Vs time in months



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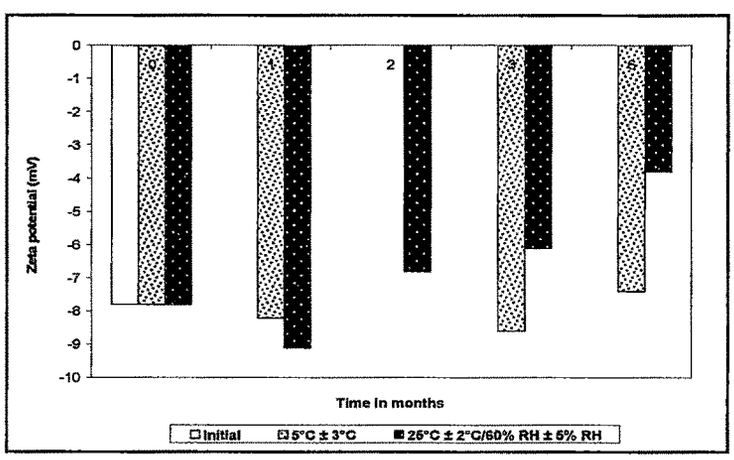

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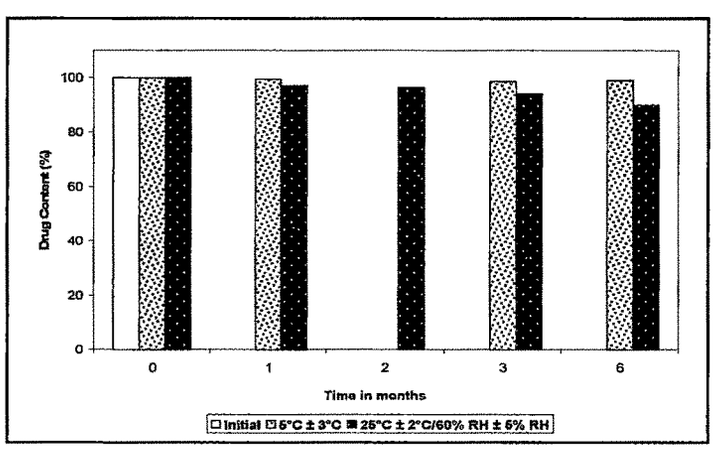
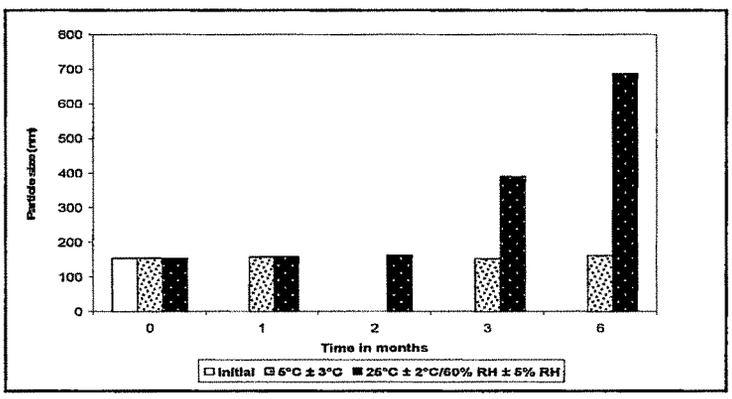
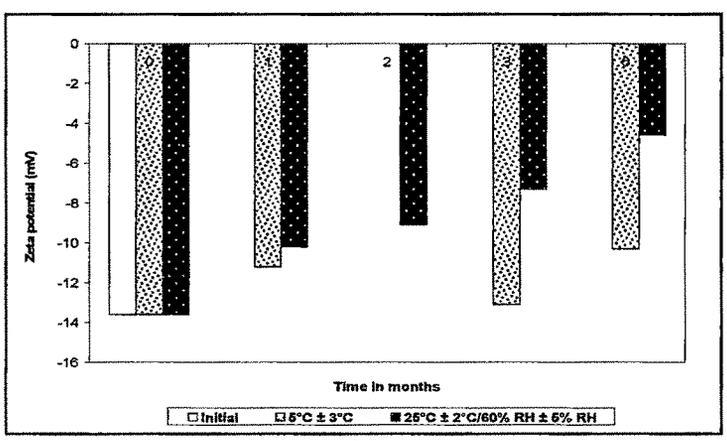


(c)

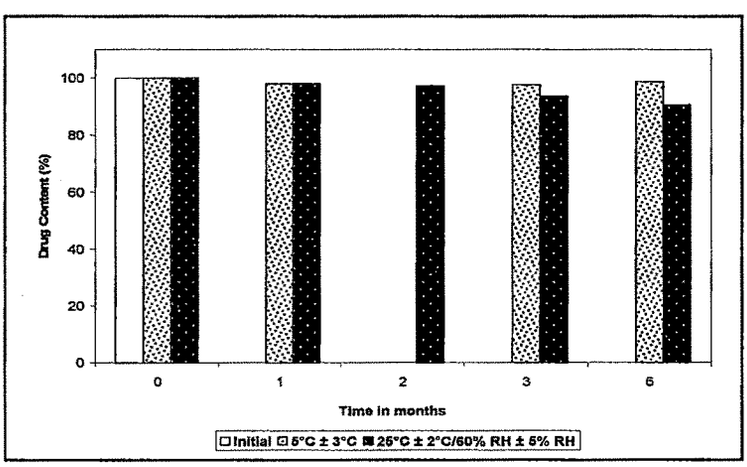
Figure 6.4 Stability profiles- Tf-PLGA-TMZ-NP (a) particle size (b) zeta potential and (c) drug content Vs time in months



(a)



(b)



(c)

It was observed that unconjugated and conjugated nanoparticles of both Etoposide and Temozolomide there was 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 unconjugated and conjugated nanoparticles of Etoposide and Temozolomide 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 nanoparticle stored at 25°C ± 2°C/60% ± 5% RH was found to increase as compared to the initial. The increase 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 Tf conjugated nanoparticles demonstrated difference in the color than the initial powder. At 6 months the color of the powder was light pink. This could be indicative of the degradation of the surface transferrin.

At 25°C ± 2°C/60% ± 5% RH, the zeta potential of the nanoparticles shifted towards the zero for both unconjugated and conjugated nanoparticles. This may be due to the acidic conditions produced due to the degradation of PLGA into lactic and glycolic acid (Sanjeeb K. Sahoo et al, 2002). The lowered zeta potential values also might have contributed toward the aggregation of particles.

The drug content of the unconjugated and conjugated nanoparticles was not altered upto 6M at 5°C ± 3°C. However, the drug content was reduced after 6M storage at 25°C ± 2°C/60% RH ± 5% RH. The drug content for Temozolomide nanoparticles was found to have significant impact, with the drug content reducing below 95% after 3M and 6M storage at 25°C ± 2°C/60% ± 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 6M samples of unconjugated and conjugated nanoparticles stored at 5°C ± 3°C were evaluated for drug release. The results for the drug release of Etoposide and Temozolomide nanoparticles are shown in figure 6.5 and 6.6 respectively.

Figure 6.5 Comparative release profile of Etoposide nanoparticles after 6M at 5°C ± 3°C

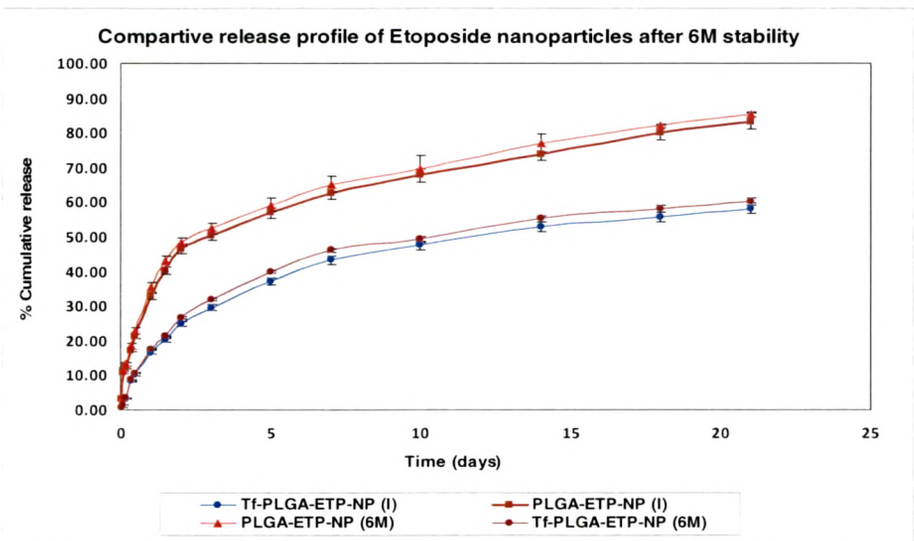
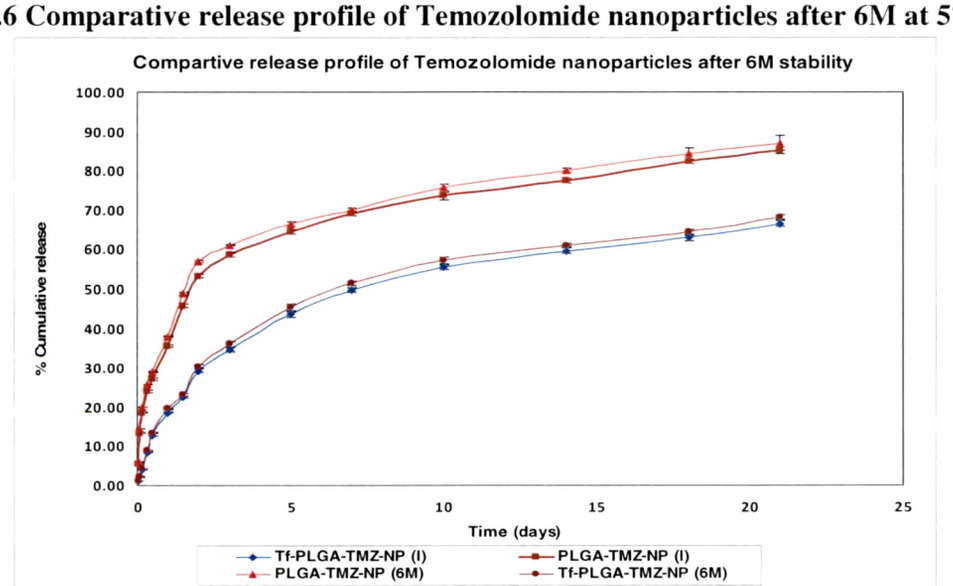


Figure 6.6 Comparative release profile of Temozolomide nanoparticles after 6M at 5°C ± 3°C



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.

6.4 Conclusions

From the above study, we can conclude that the unconjugated and Tf-conjugated PLGA nanoparticles of Etoposide and Temozolomide 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 unconjugated and conjugated nanoparticles of Etoposide and Temozolomide were stable and can be stored 5°C ± 3°C for 6M retaining its original formulation characteristics. Further, long term stability should be carried our further to assess the influence of the increasing time on the stability of the prepared nanoparticles at 5°C ± 3°C.

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