7.1. Stability Studies of Nanoparticles

Stability is one of the critical aspects in ensuring safety and efficacy of drug products. In intravenously administered nanosuspensions, for example, formation of larger particles could lead to capillary blockade and embolism so drug particle size and size distribution need to be closely monitored during storage.(1) The stability issues of drug nanoparticles could arise during manufacturing, storage and shipping. For instance, the high pressure or temperature produced during manufacturing can cause crystallinity change to the drug particles. Storage and shipping of the drug products may also bring about a variety of stability problems such as sedimentation, agglomeration and crystal growth. Therefore, stability issues associated with drug nanocrystals deserve significant attention during pharmaceutical product development.

Comparative stability studies were carried out for the potential Nanoparticle formulations at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (2-8°C) up to six months. Nanoparticle formulations were filled into type-1 tubular glass vials, purged with nitrogen, sealed and stored at the above mentioned condition.(2,3) At each sampling time different vial was used for the stability testing. The Nanoparticle formulations were examined visually for the evidence of discoloration. The content of the vials were tested for percentage drug, particle size and zetapotential.

7.2. Results and Discussion

The physical stability of nanoparticles is one of the biggest obstacles in formulation commercially viable product.(1) Nanoparticles should be stable for 1-2 years preferably at room temperature or refrigerated condition. Whichever is storage temperature, to be pharmaceutically acceptable, with high conjugation retention within nanoparticle surface and the particle size should be maintained during storage time. Hence the dissociation of antibody from nanoparticles surface, particle size growth, change in zeta-potential and the chemical stability of cetuximab were studied at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (2-8°C) up to six months. No significant differences ($p > 0.05$) were found in all above mentioned parameters at refrigerated condition. The stability results are summarized in **Table 7.1.**

7.2.1 Stability Testing Docetaxel Nanoparticles

The stability testing of prepared Docetaxel Nanoparticles was performed at accelerated condition (25°C ± 2°C, 60% RH ± 5% RH) for six months and at long-term conditions (2-8°C) up to six months and the effect on various parameters was studied. The stability testing 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. It was observed that for unconjugated and antibody conjugated Docetaxel nanoparticles, no significant change ($P>0.05$) was observed in particle size, zeta potential and drug content at 5°C ± 3°C for 6M.

At both accelerated and refrigerated conditions, Assay values were found to be within range (95-105% of initial) and change was non-significant ($p>0.05$). It was observed that for unconjugated and antibody conjugated Docetaxel nanoparticles, no significant change ($P>0.05$) was observed in particle size, zeta potential and drug content at 5°C ± 3°C for 6M.

Table 7.1 Stability Testing Data of Docetaxel Nanoparticles*

Stability Time Points	Description & Redispersibility	% Assay	% Water content	Particle size (d.nm)	Zeta Potential (mV)	% Assay	% Water content	Particle size (d.nm)	Zeta Potential (mV)
		Docetaxel Nanoparticles				Cetuximab conjugated Docetaxel Nanoparticles			
Initial	Free flowing white powder with easy redispersibility	102.5 ± 1.3	2.21 ± 0.4	178.6 ± 5.2	-27.8 ± 4.8	100.2 ± 2.3	3.11 ± 1.2	192.3 ± 6.5	-29.1 ± 3.7
5°C ± 3°C									
1 M	Free flowing white powder with easy redispersibility	99.6 ± 0.8	2.21 ± 0.4	185.2 ± 4.9	-28.2 ± 2.5	98.7 ± 1.5	3.58 ± 1.9	179.7 ± 7.9	-25.2 ± 1.8
2 M	Free flowing white powder with easy redispersibility	100.2 ± 0.5	2.65 ± 0.9	180.6 ± 7.5	-28.9 ± 3.4	101.0 ± 1.2	3.87 ± 0.7	187.6 ± 4.3	-24.5 ± 2.4
3 M	Free flowing white powder with easy redispersibility	98.9 ± 2.1	2.98 ± 1.3	187.4 ± 7.1	-23.7 ± 3.5	99.5 ± 3.3	3.92 ± 1.1	196.6 ± 2.5	-21.6 ± 2.8
6 M	Free flowing white powder with easy redispersibility	100.1 ± 1.7	3.51 ± 1.2	188.2 ± 4.5	-21.5 ± 2.1	98.6 ± 3.1	4.08 ± 2.5	201.5 ± 7.8	-21.3 ± 2.2
25°C ± 2°C/60% RH ± 5% RH									
1 M	Free flowing white powder with easy redispersibility	99.5 ± 0.8	2.87 ± 1.1	188.6 ± 5.7	-26.8 ± 1.8	99.8 ± 3.1	3.69 ± 2.1	189.7 ± 4.1	-24.8 ± 2.2
2 M	Free flowing white powder with easy redispersibility	99.1 ± 2.7	3.10 ± 0.8	197.6 ± 3.9	-22.6 ± 3.7	101.1 ± 2.7	4.11 ± 1.4	207.5 ± 4.1	-21.8 ± 1.6
3 M	Poorly flowing white powder with poor redispersibility	100.2 ± 1.3	3.25 ± 1.2	201.5 ± 7.8	-21.8 ± 2.8	97.8 ± 1.8	4.03 ± 2.3	221.4 ± 5.3	-17.9 ± 3.1
6 M	Poorly flowing white powder with poor redispersibility	98.5 ± 2.2	3.66 ± 1.0	214.8 ± 5.9	-17.5 ± 2.4	94.2 ± 3.0	4.51 ± 2.7	234.2 ± 6.1	-11.7 ± 1.9

*Results are expressed as mean±stadard deviation (n=3).

The storage of the unconjugated and antibody conjugated drug nanoparticles of the drugs at 25°C ± 2°C/60% ± 5% RH, led to an 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. 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.(3–6)

Water content was increased at accelerated condition while refrigerated condition maintained the water content value even after six months of storage.

At 25°C ± 2°C/60% ± 5% RH, the zeta potential of the nanoparticles shifted towards zero for both unconjugated and antibody conjugated drug nanoparticles. This may be due to the acidic conditions produced due to the degradation of PLGA into lactic and glycolic acid (Sahoo et al 2002). The lowered or increased zeta potential values also might have contributed toward the aggregation of particles. The drug content of the unconjugated and antibody conjugated drug nanoparticles was not changed at 6M at 5°C ± 3°C and, the drug content was reduced after 6M storage at 25°C ± 2°C/60% RH ± 5% RH. The drug content for docetaxel nanoparticles was found to have significant impact, with the drug content reducing below 95% after 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. Thus the unconjugated and antibody conjugated PLGA nanoparticles when stored at 25°C ± 2°C/60% RH ± 5% RH for 6M show instability reflected by change in physical appearance, poor flow and redispersibility and reduction in the drug content. Hence, we can conclusively specify that both unconjugated and antibody conjugated nanoparticles of the three drugs were stable and can be stored 5°C ± 3°C for 6M retaining its original formulation characteristics.(1,5,7,8)

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