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Stability Study of Chitosan Nanoparticles Containing Some Antiretroviral Drugs

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ABSTRACT

The objective of the present work was to study the stability of the prepared chitosan nanoparticles containing antiretroviral drugs like Lamivudine, Zidovudine and Stavudine. The nanoparticles were prepared by ionic gelation method using TPP as the cross linking agent. The prepared nanoparticles were characterized for particle size distribution, percent drug content, entrapment efficiency, Fourier Transform Infrared Spectroscopy, differential scanning calorimetry, scanning electron microscopy and *in vitro* dissolution studies. The prepared nanoparticles were filled in hard gelatin capsules and were subjected for long-term stability studies and accelerated stability studies. Long-term stability studies were carried out for the selected ideal batches FL-4, FZ-2 and FS-5 at $5^{o}C \pm 3^{o}C$ and $30^{o}C \pm 2^{o}C$, 65% ± 5 % RH. The samples were stored at the above said condition for minimum 1 year and their drug content and *in vitro* release was determined for every 3 months. Similarly an accelerated stability study was carried out by storing the selected preparationsFL-4, FZ-2 and FS-5 at $40^{o}C \pm 2^{o}C$, 75% ± 5 % RH for about 6 months. The drug content and in vitro release were determined for every 3 months (ICH Guidelines).

Keywords: antiretroviral drugs,ionic gelation method,stability studies, chitosan

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INTRODUCTION

Human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS) commonly referred to as HIV&AIDS have emerged as being amongst the most serious and challenging public health problems in the world. There are two species of HIV, namely, HIV 1 and HIV 2 with their respective subspecies. HIV 1 is the global common infection whereas the latter is restricted to mainly West Africa. The current clinical therapy, known as highly active antiretroviral treatment (HAART), is considered as one of the most significant advances in the field of HIV therapy. HAART is a lifelong necessity and any non-compliance leads to a rapid increase in the viral load. The reason for this relapse is related to the poor targeting ability of the antiretroviral agent to the latent sites of infection [1].Furthermore several antiretroviral drugs suffer from low bioavailability due to extensive first-pass effects and gastrointestinal degradation. In addition, for most drugs the half-life is short, thus necessitating frequent administration of doses thereby decreasing patient compliance and increasing side effects due to peak-trough fluctuations. Current attempts to overcome these limitations include the identification of new chemical identities, the examination of various dosing regimens, as well as the development of novel drug delivery systems that can improve the efficacy of both existing and new antiretroviral drugs.

Conventional antiretroviral (lamivudine, Zidovudine and Stavudine) formulations are administered multiple times a day because of its moderate half-life.Treatment of AIDS using conventional formulations of antiretroviral is found to have many drawbacks, such as adverse side effects resulting from accumulation of drug in multidose therapy, poor patient compliance, and high cost [2]. One of the suitable methods to overcome these problems could be association with biodegradable polymeric carriers such as nanoparticles. The nanometric size of these carrier systems allows efficient crossing of biological barriers, amelioration in tissue tolerance, improved cellular uptake and transport, thus enabling efficient delivery of the therapeutic agents to the target sites like liver, brain and solid tumor [3-5]. Nanoparticles may become one of the successful carriers by overcoming problems caused by infections that are refractory to conventional treatment. Chitosan possesses some ideal properties of a polymeric carrier for nanoparticles such as biocompatibility, biodegradability, non-toxicity, and low cost. It possesses a positive charge and exhibits an absorption enhancing effect. This characteristic can be employed to prepare cross-linked chitosan nanoparticles [6]Chitosan nanoparticles containing antiretroviral drugs like lamivudine, Zidovudine and Stavudine by ionic gelation technique using TPP as the cross linking agent. In the present study, stability of chitosan nanoparticles was investigatedaccording to the ICH guidelines. Drug content, sizeand *in vitro* release of nanoparticles were evaluated during the storage period of stability studies.

MATERIALS AND METHODS

Drugs such as lamivudine, Zidovudine and Stavudine used were a gift sample from Cipla Pvt. Ltd. Mumbai and chitosan from Central Institute of Fisheries Technology, Cochin, India. Glacial acetic acid and sodium tripolyphosphate were obtained from Merck Specialties Private Limited, Mumbai, India. All other chemicals used were of analytical grade.

Experimental methods

Chitosan nanoparticles containing antiretroviral drugs were prepared by ionic cross linking of chitosan solution with TPP anions.Chitosan polymer concentration is varied from 10-50 mg by keeping the drug concentration constant 10 mg. Likewise for all three drugs fifteen formulations were made among those, ideal formulations were selected for stability studies. Based on drug content, particle size, zeta potential, *in vitro* release formulation FL-4 FZ-2 and FS-5 were selected as the ideal batch from the prepared chitosan nanoparticles containing Lamivudine, Zidovudine and Stavudine respectively and that were selected for stability studies. The nanoparticles prepared in the present study were filled in the hard gelatin capsules and then filled in HDPE containers and stored at the following conditions like $5^{o} \pm 3^{o}$ C, $30^{o} \pm 2^{o}$C, 65% ± 5% RH (long term stability), $40^{o} \pm 2^{o}$C, 75% ± 5% RH (accelerated stability) [7]. Every three months the drug content and *in vitro* release were determined for samples subjected for long term stability studies and accelerated stability studies. The drug content and in vitro release were determined for every 3 months (ICH Guidelines) as per the following methods.

Drug content [8]

Drug content was determined by centrifugation method. The re-dispersed nanoparticles suspension was centrifuged at 15,000 rpm for 40 min at 25^{o}C to separate the free drug in the supernatant. Concentration of the drug in the supernatant was determined by using UV-Visible spectrophotometer at their respective λ max after suitable dilution.

In vitro release studies [9, 10]

In vitro release studies were carried out by using a cellulose dialysis bag (cutoff 5 kDa, Himedia, India). The stored nanoparticles were re-dispersed in 5ml of phosphate buffer pH 7.4 and subjected to dialysis by immersing the dialysis tube to the receptor compartment containing 150 ml of phosphate buffer pH 7.4. The medium in the receptor was agitated continuously using a magnetic stirrer and the temperature was maintained at 37 ± 1^{o}C. 5ml sample of the receptor compartment was taken at various intervals of time over a period of 24 hours and each time 5 ml fresh buffer was replaced. The amount of drug released was determined spectrophotometrically at their respective λ maxfor every three months interval.

RESULTS AND DISCUSSION

On comparing the drug content after storing the nanoparticles FL-4,FZ-2 and FS-5 at $5^{o}\pm3^{o}$ Cand$30^{o}\pm2^{o}$C, 65% ± 5% RH with the previous data it was observed that there was a slight decrease in the drug content after 12 months of storage (Fig 1, 2, 4, 5, 7, 8). Every three months the *in vitro* release almost remains the same for nanoparticles FL-4,FZ-2 and FS-5 stored at $5^{o}\pm 3^{o}$ C and$30^{o}\pm2^{o}$C, 65% ± 5% RH up to 12 months (Table 1, 2 and 3). However, the nanoparticlesFL-4,FZ-2 and FS-5, which were subjected for accelerated stability studies at $40^{o} \pm 2^{o}$C, 75% ± 5% RH shows a significant decrease in, drug content (Fig 3, 6, 9) and *in vitro* release(Table 1, 2, 3) . It may be due to the reason that at high temperature there might be chances for drug degradation that decrease the drug content

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and thereby decrease the drug release percentage. SEM of formulationsFL-4,FZ-2 and FS-5 stored at 40°±2°C, 75%± 5%RH shows increased particle size after 6 months of storage (Fig 10, 11, 12). This may be due to agglomeration of particles. Therefore from this stability studies it was observed that the prepared nanoparticles will be stable in 5°± 3° C and30°±2°C, 65% ± 5%RH for a period of 12 months.

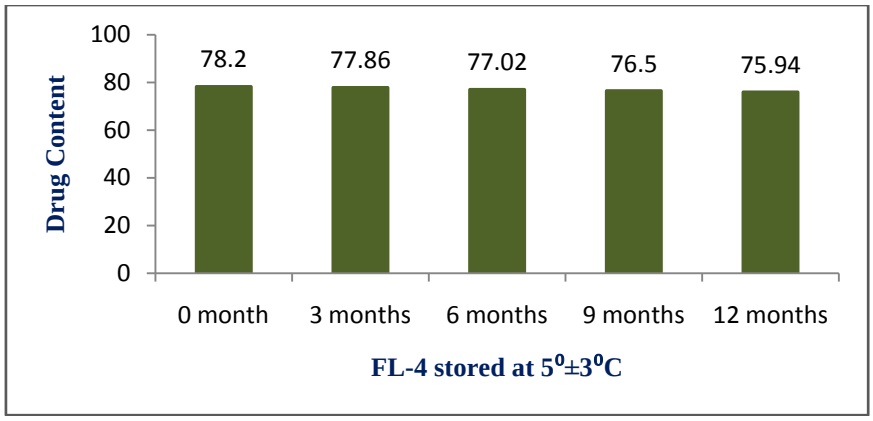

Fig 1 Comparison of percentage drug content of formulation FL-4 stored at 5° ± 3° C, (after 0,3,6,9 &12 months storage as per QA1(R))

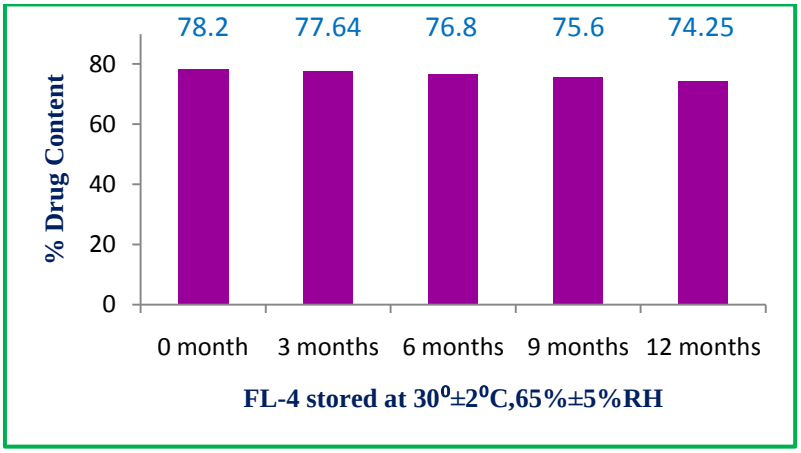

Fig 2 Comparison of percentage drug content of formulation FL-4stored at30°± 2°C, 65% ± 5% RH after 0,3,6,9 &12 months storage (as per QA1(R))

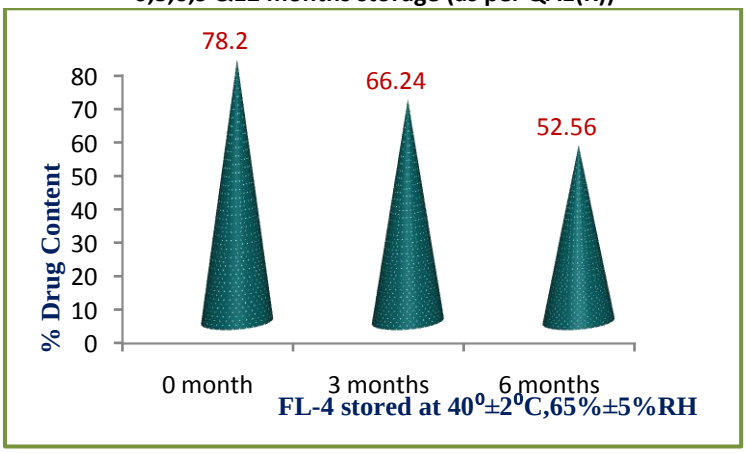

Fig 3 Comparison of percentage drug content of formulation FL-4 stored at 40° ± 2°C, 75% ± 5% RH (after 0, 3, & 6 months storage)

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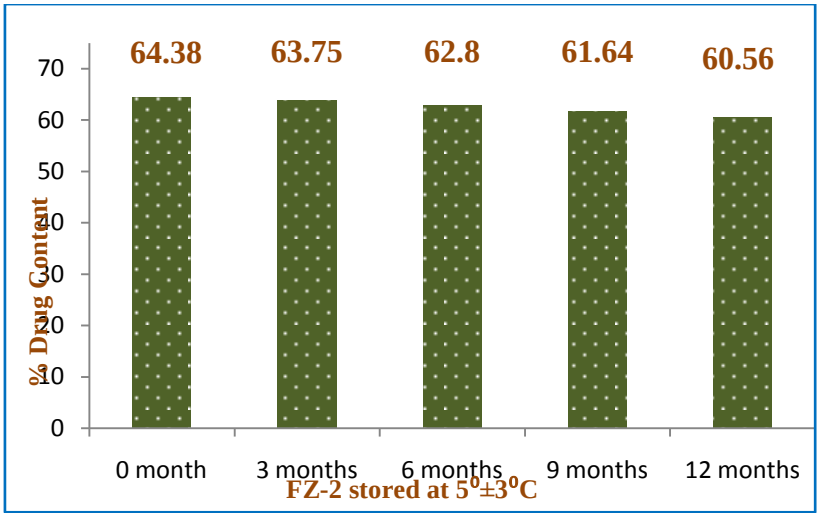

Fig 4 Comparison of percentage drug content of formulation FZ-2 stored at 5° ± 3° C, (after 0,3,6,9 &12 months storage as per QA1(R))

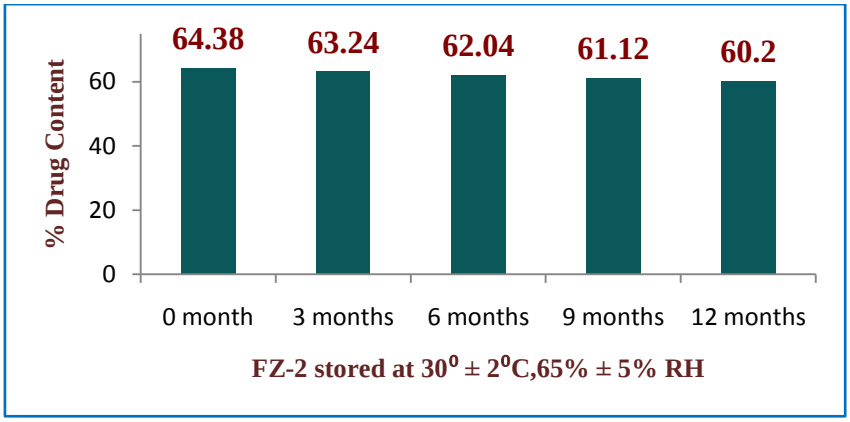

Fig 5 Comparison of percentage drug content of formulation FZ-2 stored at 30° ± 2°C, 65% ± 5% RH after 0,3,6,9 &12 months storage (as per QA1(R))

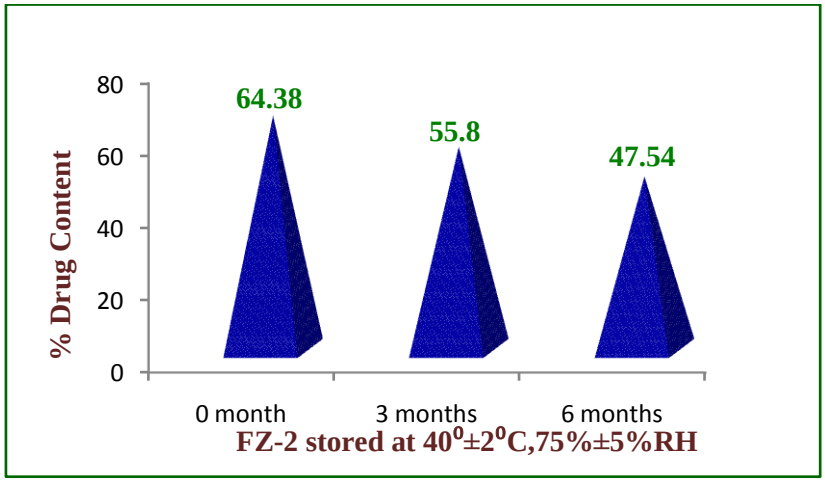

Fig 6 Comparison of percentage drug content of formulation FZ-2 stored at 40° ± 2°C, 75% ± 5% RH (after 0, 3, & 6 months storage)

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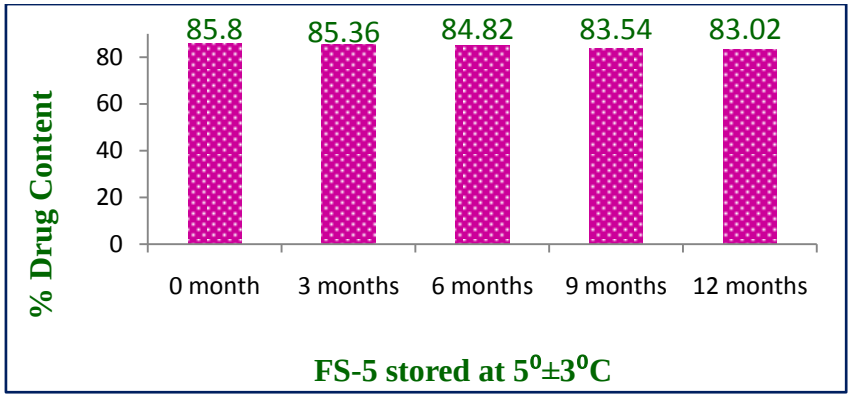

Fig 7 Comparison of percentage drug content of formulation FS-5 storedat 5° ± 3° C, (after 0,3,6,9 &12 months storage as per QA1(R))

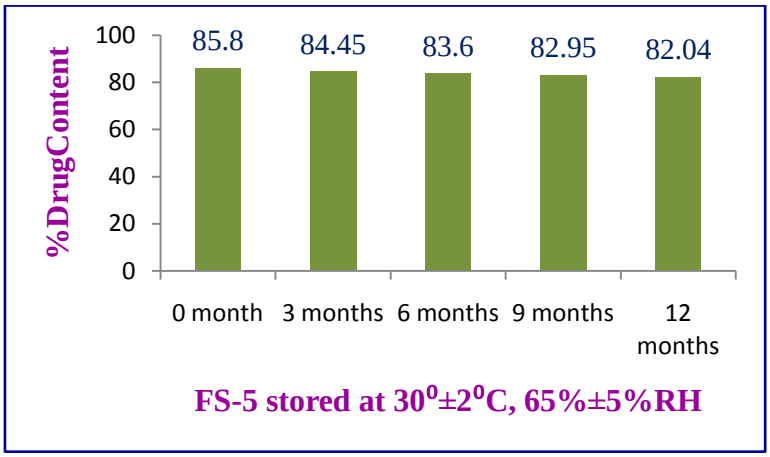

Fig 8 Comparison of percentage drug content of formulation FS-5 stored at30° ± 2°C, 65% ± 5% RH after 0,3,6,9 &12 months storage (as per QA1(R))

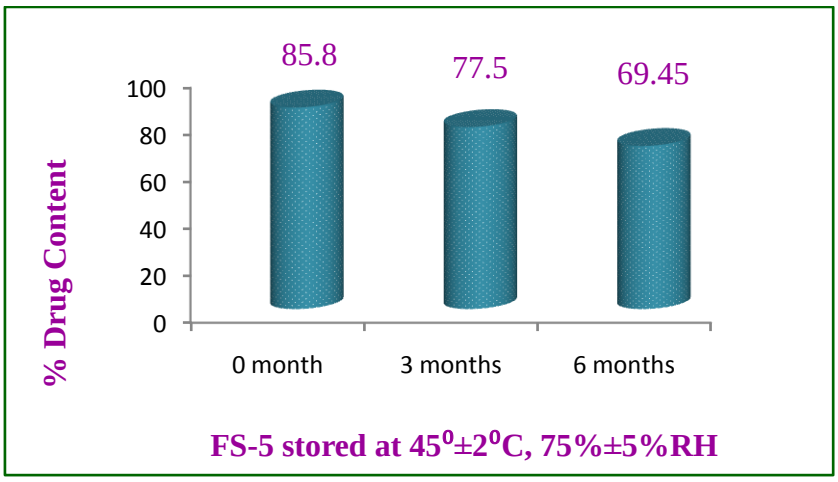

Fig 9 Comparison of percentage drug content of formulation FS-5 stored at 40° ± 2°C, 75% ± 5% RH (after 0, 3, & 6 months storage)

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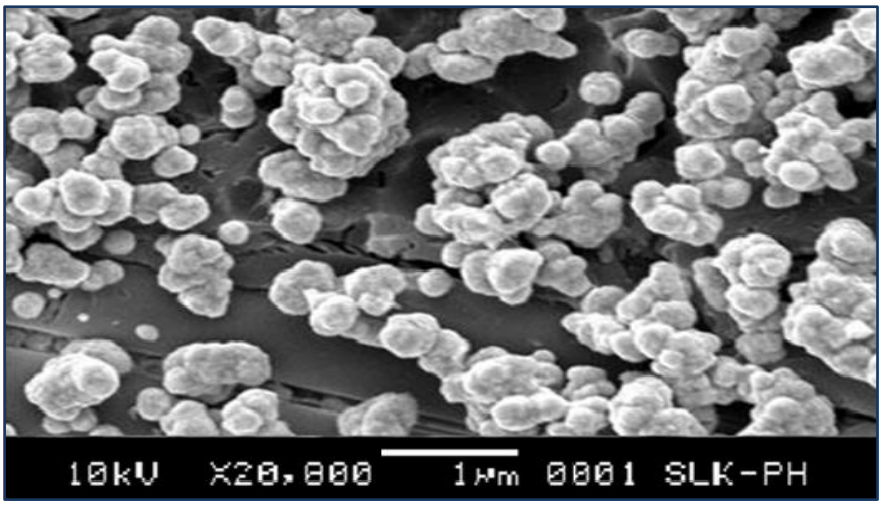

Fig 10 SEM of formulation FL-4 at 40º ± 2ºC, 75% ± 5% RHafter6 months storage

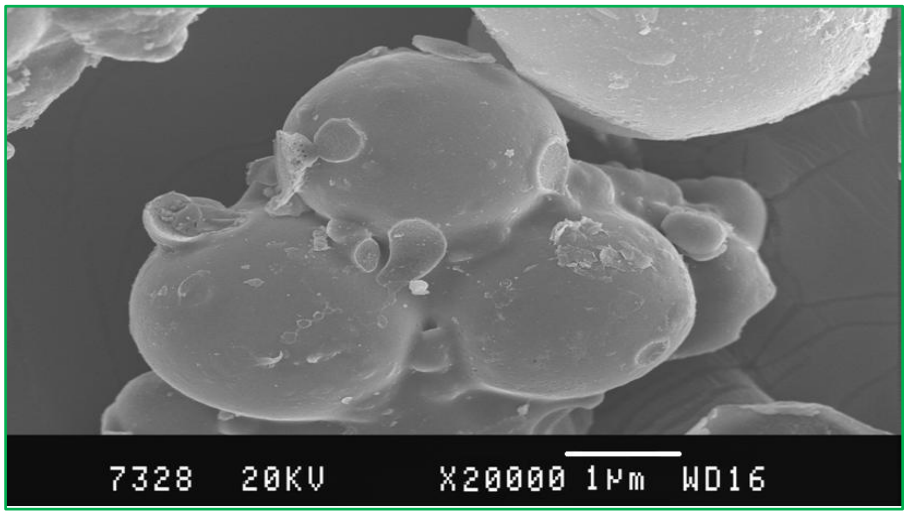

Fig 11 SEM of formulation FZ-2 at 40º ± 2ºC, 75% ± 5% RH after 6 months storage

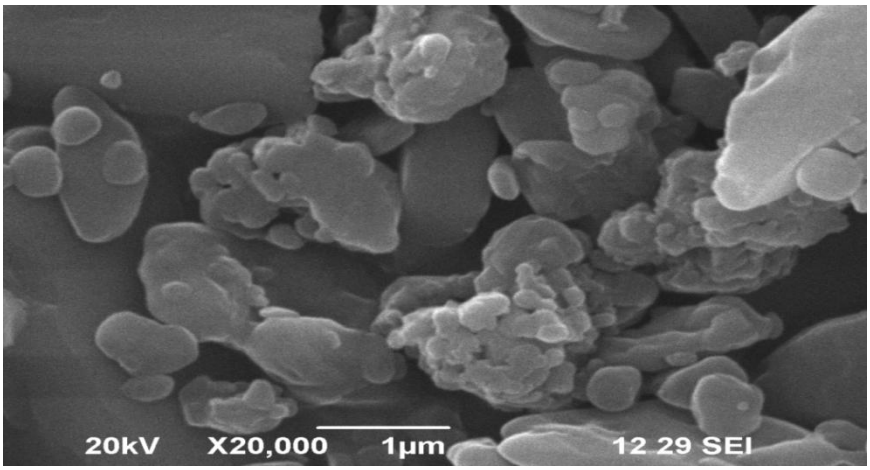

Fig 12 SEM of formulation FS-5 at 40º ± 2ºC, 75% ± 5% RH after 6 months storage

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Table 1 Stability studies- *In vitro* release of FL-4 stored at 5° ± 3°C, 30° ± 2°C, 65% ± 5% RH, 40° ± 2°C, 75% ± 5% RH (after 0, 3, 6 & 9 months storage)

Time (Hrs)	% Cumulative drug release												
	5° ± 3°C					30° ± 2°C, 65% ± 5% RH					40° ± 2°C, 75% ± 5% RH		
	0	3	6	9	12	0	3	6	9	12	0	3	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	22.9	22.74	22.62	21.84	20.44	22.9	22.49	22.46	21.54	19.68	22.9	20.92	17.78
2	24.9	24.24	24.16	23.26	21.6	24.9	23.96	23.78	22.05	20.84	24.9	21.87	19.56
3	29.3	28.98	28.79	27.35	25.74	29.3	28.34	28.26	26.76	24.57	29.3	27.28	24.43
4	32.2	32.75	32.64	30.96	27.85	32.2	32.23	32.16	30.45	28.05	32.2	30.28	27.96
6	37.7	37.67	37.48	36.15	34.76	37.7	37.76	37.58	36.25	34.76	37.7	36.74	33.76
8	42.7	42.17	42.05	41.75	39.68	42.7	41.88	41.72	40.58	38.82	42.7	40.37	37.06
10	47.2	46.78	46.58	45.84	43.92	47.2	46.29	46.25	44.96	43.28	47.2	44.23	42.23
12	51.6	51.19	51.06	50.42	48.26	51.6	50.93	50. 78	49.04	47.74	51.6	48.74	44.65
24	79.5	79.2	79.16	78.48	76.95	79.5	77.9	77.84	76.55	75.25	79.5	69.1	58.86

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Table 2 Stability studies- *In vitro* release of FZ-2 stored at 5° ± 3°C, 30° ± 2°C, 65% ± 5% RH, 40° ± 2°C, 75% ± 5% RH (after 0, 3, 6 & 9 months storage)

Time (Hrs)	% Cumulative drug release												
	5° ± 3°C					30° ± 2°C, 65% ± 5% RH					40° ± 2°C, 75% ± 5% RH		
	0	3	6	9	12	0	3	6	9	12	0	3	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	22.9	22.56	21.26	20.58	19.62	22.9	20.29	19.45	18.68	16.46	22.9	20.29	17.29
2	24.87	24.48	24.08	23.84	22.55	24.87	24.12	23.84	22.95	20.06	24.87	21.27	19.27
3	29.34	28.94	28.24	27.56	26.78	29.34	28.38	27.34	26.25	23.74	29.34	25.35	23.35
4	32.2	31.85	31.32	30.95	29.04	32.2	31.12	30.04	29.85	25.62	32.2	28.26	26.26
6	37.7	37.02	36.47	35.64	33.78	37.7	36.7	36.26	34.67	30.28	37.7	33.87	30.57
8	42.7	42.02	41.52	40.36	38.56	42.7	41.07	40.45	38.38	35.45	42.7	38.27	34.67
10	43.8	43.14	42.42	40.86	37.95	43.8	42.98	41.56	39.52	36.68	43.8	39.8	36.68
12	45.54	44.76	43.14	41.28	40.26	45.54	44.24	43.78	41.94	38.65	45.54	41.54	38.24
24	72.06	71.18	70.68	69.54	67.45	72.06	71.26	70.54	68.78	66.36	72.06	64.36	59.26

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Table 3 Stability studies- *In vitro* release of FS-5 stored at 5° ± 3°C, 30° ± 2°C, 65% ± 5% RH, 40° ± 2°C, 75% ± 5% RH (after 0, 3, 6 & 9 months storage)

Time (Hrs)	% Cumulative drug release												
	5° ± 3°C					30° ± 2°C, 65% ± 5% RH					40° ± 2°C, 75% ± 5% RH		
	0	3	6	9	12	0	3	6	9	12	0	3	6
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	20.45	20.35	19.85	18.76	18.28	20.45	20.02	19.42	17.65	14.45	20.45	18.02	16.02
2	25.63	25.03	24.74	24.22	23.75	25.63	24.68	23.88	21.86	19.55	25.63	21.63	19.63
3	30.52	29.99	29.68	28.54	27.82	30.52	29.05	28.75	26.02	24.82	30.52	25.34	23.34
4	35.72	35.02	34.52	33.86	31.64	35.72	36.53	34.96	32.42	29.64	35.72	30.56	27.56
6	42.28	41.16	40.16	39.48	37.53	42.28	41.82	40.65	38.66	36.32	41.28	36.72	31.72
8	49.52	49.02	48.62	47.34	45.86	49.52	48.96	47.96	45.12	42.76	49.52	43.54	39.58
10	55.15	54.92	54.32	53.66	52.62	55.15	55.02	53.92	50.86	48.38	55.15	48.94	44.05
12	61.56	60.45	60.26	58.95	57.34	61.56	60.28	59.46	56.75	54.05	62.06	54.79	49.78
24	76.74	76.12	75.42	74.04	73.5	76.74	75.86	74.86	73.78	71.05	76.74	70.65	65.62

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