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Research Article

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Chitosen based Nano structured Lipid Carrier of Acyclovir

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Abstract The investigation was to improve the bioavailability of acyclovir by incorporating it into solid lipid nanoparticles (SLNs) and chitosen nano structured lipid carriers (NLCs). The aim of present study is to develop chitosan nanoparticle formulation in order to achieve triggered release of anticancer drug encapsulated in chitosan nanoparticle. Evaluation for the potential of novel chitosan nanoparticle for enhanced cytotoxic effects and minimum side effects. The chitosan nanoparticle was prepared according to the ionic gelation method. Using TPP as cross linker with slight modification. After a complete dissolution of chitosan in acetic acid, gamma oryganol (5%) was added to this solution with magnetic stirring. Then TPP (0.5%) was added in drop wise through a syringe at a uniform rate. In this method glacial acetic acid 1.6 % dissolved in distilled water and further chitosan was used in various concentration % (0.25, 0.5, 1:1, 1:5:1, and 2:1). We have replace ascorbic acid with glacial acetic acid. Ratio of chitosan to TPP was varied from 0.25, 0.5, and 1:1, 1:5:1, and 2:1 % with acetic acid (1.6%) and TPP (0.5%) were used.

Keywords Chitosen, Lipid Carrier, Acyclovir

Introduction

Nano- particles (NPs) are solid particle and particulate dispersion of the drug carrier which is a simply means dwarf. The term nanoparticle is a combined form of both that may or may not be biodegradable. In the nanoparticle drug is entrapped, encapsulated, attached or dissolve in to a nanoparticle matrix. The word 'Nano' is derived from Greek word Nano-spheres and Nano capsules. Drug is compressed to a cavity enclosed by an individual polymer membrane called Nano capsules, while Nano spheres are matrix systems in which the drug is physically and undeviating dispersed. The term nanoparticle is a combined form of both Nano-spheres and Nano capsules. Where conventional technique reaches their limits, nanotechnology provides opportunities for the various medical applications [1-5]. In current year, in the development of novel antibacterial agents silver nanoparticle and gold nanoparticles have attracted significant interest because of their promising applications. However, in previous report of silver nanoparticle shows toxic effects and instability of silver nanoparticle. The toxicity of the silver nanoparticle was recently aggregate with their accumulation and penetration in the mitochondrial membrane appear the impairment of mitochondrial function .Unlike silver nanoparticle and gold nanoparticles are inert, more stable, non-toxic and size controllable. A natural and synthetic polymer shows various advantage and disadvantage. Apart from the liposomal and polymeric nanoparticles iron and gold nanoparticles are also developed and it has been appeal to enlargement of new generation of drug delivery systems [6-10].

Materials and Method

Materials

Chitosan (CS) and TPP was procured from the Shreya health care, India. Gamma Oryganolwere purchased Sigma Aldrich, Mumbai, India. Any other material and solvent used as an analytical grade and distilled water used for the preparation of all solution.

Sample preparation

Preparation of gamma oryganol loaded chitosan nanoparticle

The chitosan nanoparticle was prepared according to the ionic gelation method [11]. Using TPP as cross linker with slight modification. After acomplete dissolution of chitosan in acetic acid, gamma oryganol (5%) was added to this solution with magnetic stirring. Then TPP (0.5%) was added in drop wise through a syringe at a uniform rate. In this method glacial acetic acid 1.6 % dissolved in distilled water and further chitosan was used in various concentration % (0.25, 0.5, 1:1, 1:5:1, and 2:1). It was further stirred for 2 hours followed by centrifugation for 10 min at 10000 rpm. Supernatant was discarded and pellet was re-suspended in phosphate buffer saline (PBS). The nanoparticle was collected by the centrifugation method at 10,000 rpm for 10 min and then washed with distilled water with stirring until the opalescent color was observed and stirring continued for 30 min. and the obtained pellets was washed with a distilled water at three time. The prepared nanoparticle were lyophilized and stored at 4 °C until further use [12].

Table 1: Formula of different formulations of gamma oryganol loaded chitosan nanoparticle

S. No	Name of Ingredient	F1	F2	F3	F4	F5
1.	Gamma Oryganol %	5	5	5	5	5
2.	Chitosan	0.25	0.5	1:1	1:5:1	2:1
3.	Acetic acid	1.6	1.6	1.6	1.6	1.6
4.	TPP	0.5	0.5	0.5	0.5	0.5

Standard curve of extract of gamma oryganol

In the study of drug release 100mg of drug was dissolved in the 100 ml of distilled water concentration of solution was 1000µg/ml and note it and then1 ml sample was taken and make up the volume with 10ml in the volumetric flask and the concentration of the above solution was find to be 100µg/ml sample was taken such as 1ml, 2ml, 3ml, 4ml, 5ml and make up the volume up to 10ml in volumetric flask. And the concentration of above sample was found to be 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml and 50µg/ml. and absorbance of sample were check through the UV-visible spectrophotometer in triplicate.

Table 2: Standard calibration curve of gamma oryganol

S. No.	Concentration (µg/ml)	Absorbance (nm)
1.	10	0.063
2.	20	0.125
3.	30	0.191
4.	40	0.251
5.	50	0.33

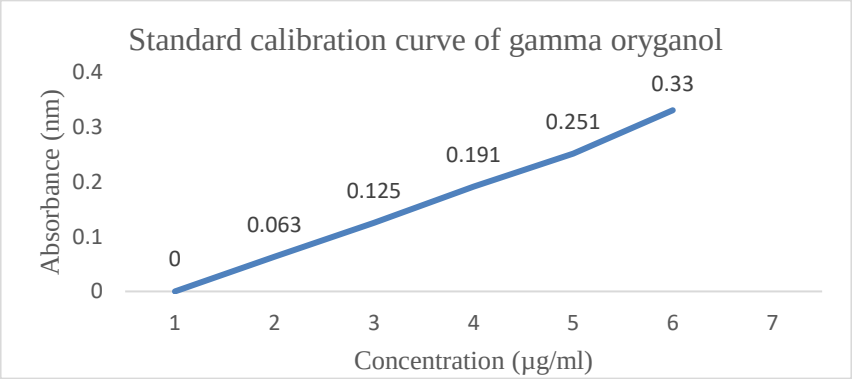

Figure 1: Standard calibration curve of gamma oryganol

Characterization of the formulated chitosan nanoparticle

The nanoparticle prepared by the ionic gelation methods, in the ionic gelation method chitosan are dissolved in glacial acetic acid was entrapped by drop wise addition of tri polyphosphate (TPP). We have replace ascorbic acid with glacial acetic acid. Ratio of chitosan to TPP was varied from 0.25, 0.5, and 1:1, 1:5:1, and 2:1 % with acetic acid (1.6%) and TPP (0.5%) were used. TPP added carefully because it forms larger particle. The formation of the larger nanoparticle because of aggregation of the nanoparticle, due to it cross-linking with each other.

Zeta potential study

The zeta potential (surface charge) of chitosan nanoparticle were analysed by zeta sizer. To determine the zeta potential of the prepared nanoparticle sample were diluted with the water (0.1ml) and it placed in the electrophoretic cell where an electrical field of 15.5 V/cm was applied. Each sample measurementwas carried out in triplicate.

Morphological studies by scanning electron microscopy

The morphology of nanoparticle was evaluated by scanning electron microscopy. In preliminary step, 100µl of the chitosan nanoparticle formulation were added in to a 10mm glass slide and dried in a vacuum desiccator at overnight in room temp. Till the SEM was performed. For the nanoparticle analysis was fixed on the adequate support and coated with the gold sputter module in a higher vacuum evaporator. Observation was taken under different magnification performed at 15kv [11-13].

Drug Encapsulation efficiency

Drug encapsulation efficiency of chitosan nanoparticle were determined by the ultra-centrifugation method. Briefly, acetic acid, and TPP with the chitosan nanoparticle were separated from the chitosan nanoparticle by using the ultra-centrifugation at 12,000rpm for 30 min. the pellet were re-dissolved in the distilled water further supernatant also scanned in this parameter with the UV-visible spectrophotometer 350nm. The drug encapsulation efficiency was determined by using the relation in this equation [14].

$$\% \text{ Drug encapsulation efficiency} = \frac{\text{Experimental drug content}}{\text{Theoretical drug content}} \times 100$$

Production yield of nanoparticle

The yield of nanoparticle was determined by comparing the whole of nanoparticle formed against the combined weight of the copolymer and drug [14].

$$\% \text{ Yield calculation} = \frac{\text{Amount of drug}}{\text{Amount of drug + polymer}} \times 100$$

***In-vitro* study of drug release**

In the *in-vitro* release study of prepared chitosan nanoparticle was observed in phosphate buffer saline (PBS) (PH 7.4) at 37 ^{0}C. The chitosan nanoparticle is placed in the dialysis bag and dialyzes against the 50ml of phosphate buffer saline with the constant stirring for 1 hrs. Aliquots were removed periodically. The volume of removed sample in PBS being replaced by fresh volume of PBS. The amount drug release was determined by the monitoring the absorbance with UV-visible spectrophotometer at 350nm [14].

Anti-oxidant activity study

Antioxidant activity study of plant extract and its prepared nanoparticle were evaluated by using the different assay and compared with anti-oxidant activity of standard compound, BHT and Ascorbic acid.

- DPPH scavenging assay
- Ferric reducing anti-oxidant power assay

Stability of Nanoparticle

The prepared nanoparticle stability studies determined by storing optimized formulation in stability chamber for 8 weeks. The samples were analyzed after a time period such as 0, 1, 2, and 8weeks for their physical appearance (According to ICH Q1A)[15].

Result and Discussion

Zeta potential study

This is mostly used for size measurement, electrophoretic mobility of protein, and zeta potential of the colloidal and nanoparticle and it is also used for the micro rheology of protein and polymer solutions.

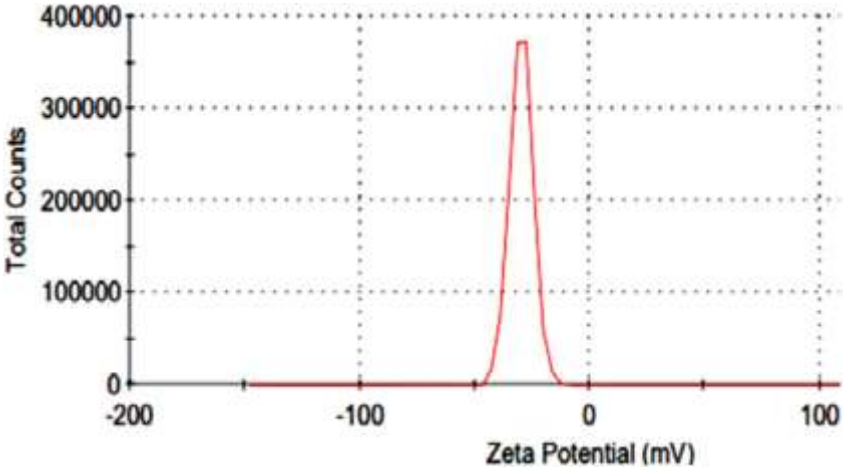

Figure 2: Zeta potential studies

Morphological studies by the scanning electron microscopy

The surface morphology of the herbal chitosan nanoparticle was investigated by scanning electron morphology. The study provide the better understanding of the morphological characteristics of the nanoparticle. There were large number of nanoparticle with an approximately spherical shape and they will separate from each other. SEM image of freeze dried gamma oryganol loaded chitosan nanoparticle at higher cross linking time, little forwarded but spherical nanoparticle with small size are obtained in F1.

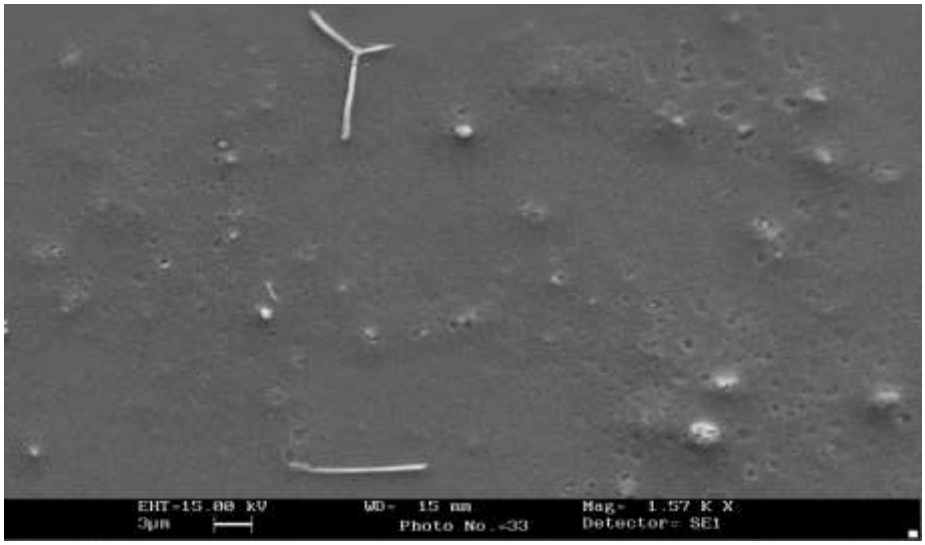

Figure 3: SEM image of F1 formulation

Encapsulation efficiency (% EE)

Entrapment efficiency of gamma oryganol loaded chitosan nanoparticle were recorded in the range between '50% to 80%' for 'F1 to F5' formulation. It is obtained at 350 nm. It revealed lowest entrapment efficiency of drug that is F4 formulation 60.84%, while F1 displayed 75.54% highest drug encapsulation efficiency and F3 and F5 formulation are also show the better formulation.

Table 3: % Encapsulation efficiency

Formulations	Encapsulation efficiency (%)
F1	75.54
F2	62.87
F3	71.23
F4	60.84
F5	71.65

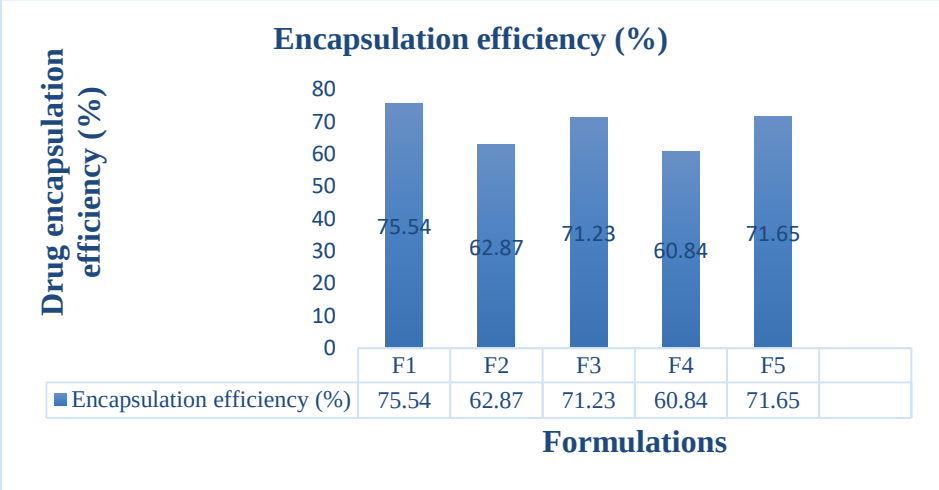

Figure 4: Graphical representation of % Encapsulation efficiency

***In-vitro* drug release studies**

In the *in- vitro* drug release study of gamma oryganol loaded chitosan nanoparticle of all formulation were noted. The maximum drug release revealed that 90.65 % of drug release from formulation F1. It was found that on increasing the concentration of the polymer, drug release from the nanoparticle decreased. The high stirring speed and high concentration of polymer make stiff nanoparticle. The more hardness of the nanoparticle decrease the drug release rate from nanoparticle, thus F1 formulation exhibited the sustained release of drug from nanoparticle in comparison of others.

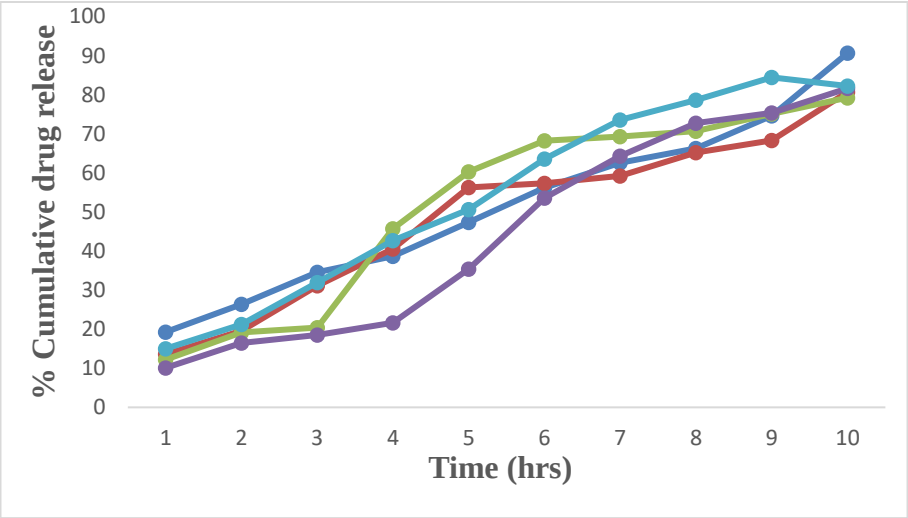

Figure 5: %Cumulative drug release of all formulation

Production yield of nanoparticle

It was recorded that as concentration of polymer increase production yield decrease, since increase in polymeric concentration make solution viscous this was difficult to pour and get sticked on the wall of the beaker. The production yield of gamma oryganol loaded chitosan nanoparticle was found to be maximum for F1 (73.59%) and it having lowest amount of polymer. The lowest % yield was of formulation F4 (51.46%) stirring rate also affect the production yield of nanoparticle.

Table 4: Production yield of all formulation

Formulations	Practical mass (mg)	Theoretical mass (mg)	Production Yield (%)
F1	73.59	100	73.59
F2	63.12	100	63.12
F3	61.24	100	73.59
F4	51.46	100	51.46
F5	54.03	100	54.03

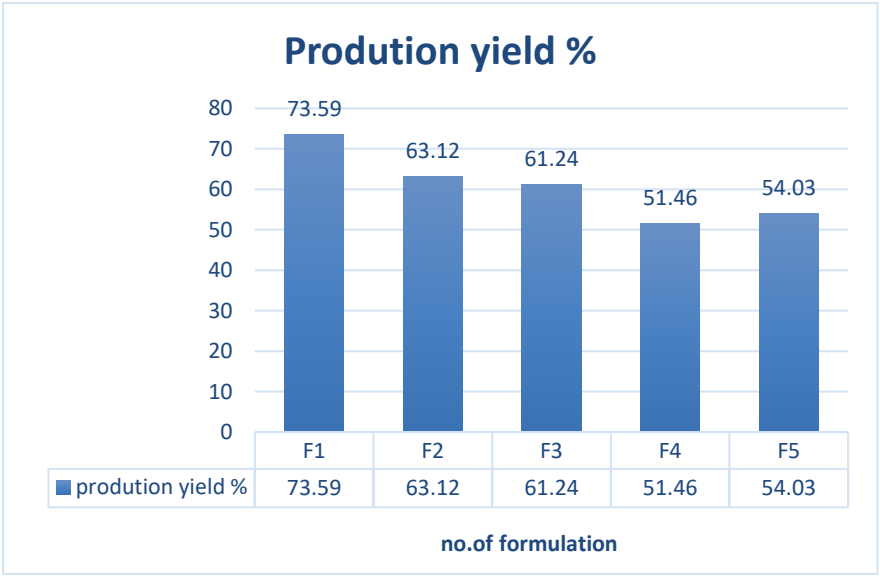

Figure 6: Graphical representation of production yield of all the formulation

Anti-oxidant activity study

DPPH scavenging assay

Table 5: DPPH scavenging assay for ascorbic acid

S. No.	Conc.	Absorbance of sample	Control	Control-sample/ control	% inhibition
1	10	0.1365	0.245	0.46875	46.873
2	20	0.128	0.245	0.49609	49.6094
3	30	0.1222	0.245	0.52358	52.3538
4	40	0.1144	0.245	0.55458	55.4588
5	50	0.111	0.245	0.57013	57.6584
6	60	0.1035	0.245	0.59796	59.7656
7	70	0.98	0.245	0.62109	62.1096
8	80	0.088	0.245	0.65188	65.2344
9	90	0.085	0.245	0.67188	67.1875
10	100	0.075	0.245	0.69921	69.3652

Table 6: DPPH scavenging assay for gamma oryzanol loaded chitosan nanoparticle

Conc.	% inhibition of ascorbic acid	% inhibition of F1	% inhibition of F2	% inhibition of F3	% inhibition of F4	% inhibition of F5
10	49.6093	46.8751	47.8851	46.8751	47.8758	48.0051
20	52.3576	49.6094	48.6094	49.6094	51.9877	52.6088
30	55.4578	52.3586	53.3586	53.3686	52.6286	54.5619
40	57.0126	55.4578	55.4578	57.0007	57.4548	59.7878
50	59.7956	57.0126	57.2313	59.0132	59.6543	60.9566
60	62.1094	59.7956	64.7956	64.1826	65.6596	65.5656
70	65.1876	62.1037	66.1067	66.1827	68.8904	68.1137
80	67.1876	65.1876	68.1876	69.9908	70.6518	70.1846
90	75.9121	67.1886	70.1886	72.6327	70.9219	75.1865
100	85.6093	69.33	75.6396	76.1331	78.3923	80.9947

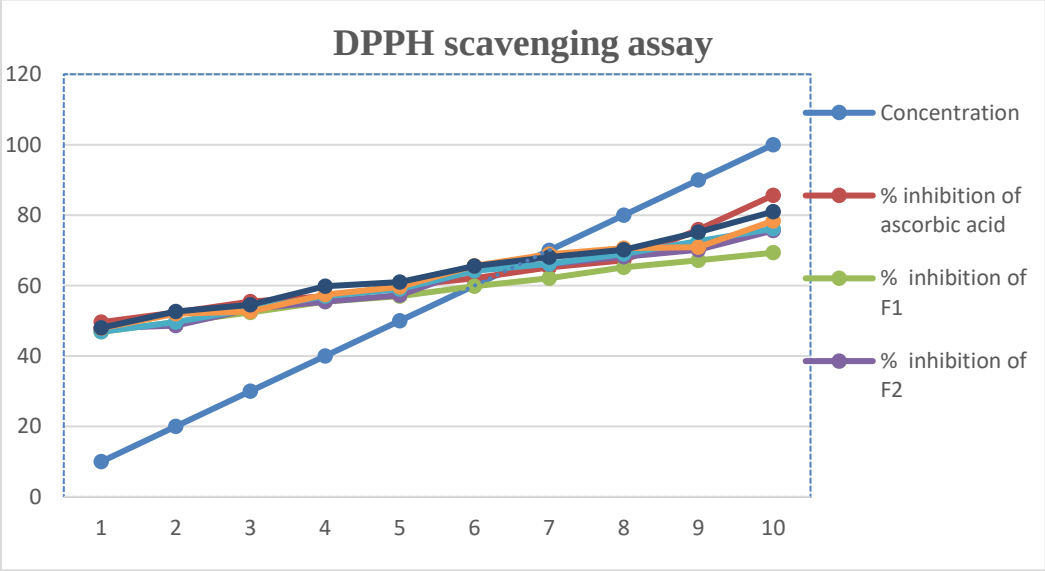

Figure 7: % DPPH Scavenging Assay

Ferric reducing anti-oxidant power assay

Table 7: Ferric reducing anti-oxidant power assay

Conc	% inhibition of ascorbic acid	% inhibition of F1	% inhibition of F2	% inhibition of F3	% inhibition of F4	% inhibition of F5
10	29.60938	26.8745	25.49845	29.89484	32.65976	35.48489
20	33.12525	33.4567	35.18451	36.18486	35.48412	38.91182
30	37.44189	36.4531	37.48941	39.49846	42.95942	42.28412
40	41.11926	40.69213	39.48451	41.48952	47.56484	45.9856
50	47.56981	42.76563	42.48963	42.65554	50.148412	50.35848
60	49.48383	45.10938	46.51112	48.54138	53.24698	53.59513
70	51.83714	50.64581	49.58412	51.66557	55.65484	58.49846
80	53.54781	54.21378	50.65742	53.54984	58.58412	61.85488
90	65.85936	62.64842	52.44415	56.96545	60.54894	63.48487
100	68.16326	70.81654	58.84841	60.56498	63.48911	65.32657

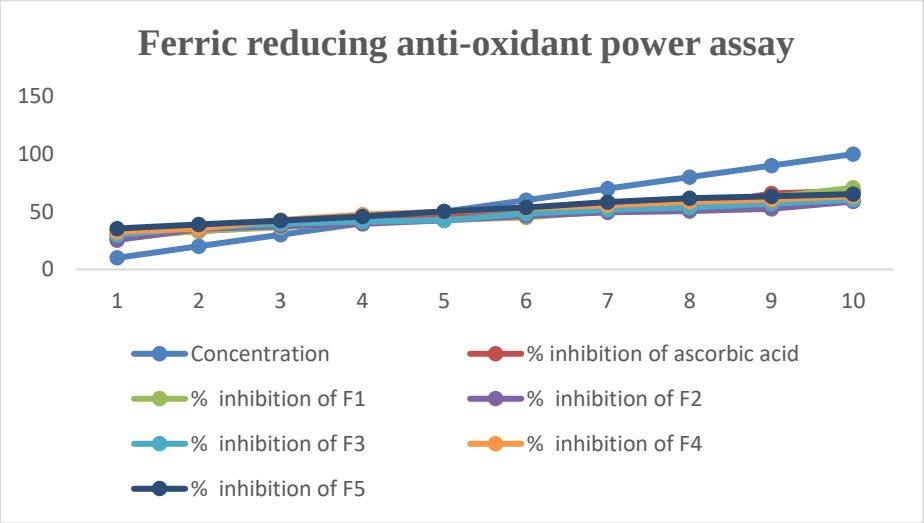

Figure 8: Ferric reducing anti-oxidant power assay

Stability study

The stability ofchitosan nanoparticle was also used for the determined by measuring its absorption spectra after 8 Weeks. There is no significant changes occur during the storage condition, the chitosan nanoparticle did not agglomerate it indicate they were more stable during the period.

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