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Formulation and Evaluation of *Abelmoschus moschatus* Nanoparticles: A Novel Approach for Encapsulation of the Hydrophobic Drug

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ABSTRACT

Nanoparticles have generated a budding interest in the number of advancements throughout recent years. Nanophytomedicines are prepared from active phytoconstituents or standardized extracts. Potential phytoconstituents isolated from the herbal source are equipotent to the synthetic drug. Despite, the therapeutic outcome is limited due to its aqueous solubility and bioavailability. Therefore it is necessary to develop a system which could provide an elevated pool for such bioactive compounds. Many such numbers of natural phytoconstituents are been used in daily life and in the pharmaceutical field. Phytosterols are the cholesterol-like molecules found in all plant foods, the highest concentrations occurring in vegetable oils. One widely known such compound is Beta-Sitosterol, which is obtained from the plant named Abelmoschus moschatus (Ranbhendi). This plant contains a chemical constituent named Beta-Sitosterol showing anti-cancer properties. The chemist called it "plant sterol ester," it is found in fruits, vegetables, nuts, and seeds. Along with its anti-cancer properties, it helps in reducing the inflammation, reducing cholesterol and is used in various heart and gastrointestinal diseases. By incorporating the drug in nanoparticles it is easy to achieve greater efficacy at a targeted site with no toxicity.

INTRODUCTION: [1,3,4,5]

Nanoparticles are defined as the particulate dispersion or solid particles in with the size range of 10-1000 nm. The drug is dissolved, entrapped and encapsulated or attached to a nanoparticle matrix. Depending upon the size they are classified accordingly. Plants have formed the major basis of a traditional system of medicine. More than 80% of the world's population depends on the traditional system or medicines to meet their goals of primary health care. The herbal treatment helps to increase the therapeutic value by reducing the toxicity and side effects of drugs at the same time it also increases the bioavailability. Nanotechnology plays a great role and the use of nanotechnology in herbal medicine and more specifically in drug delivery is set to spread rapidly. Nano herbal drug delivery systems have a potential future for enhancing the activity and overcoming the problems associated with medicinal plants. So the herbal nanocarriers help to treat the dangerous diseases like cancer, diabetes etc.

Phytosterols are the compounds found in plants that resemble cholesterol. The highest concentration of phytosterols is found naturally in vegetable oils, beans, and nuts. Phytosterols play an important role in cholesterol-lowering benefits, cancer protection, skin protection benefits changing immune system etc. Beta Sitosterol is used for heart diseases, boosting the immune system and for preventing various types of cancers like colon cancer, breast cancer, stomach cancer, leukemia etc.

Beta Sitosterol is a plant steroid derived from Abelmoschus moschatus commonly called as Ranbhendi belonging to Malvaceae family. Nanoparticles prepared from Beta Sitosterol are used for the treatment of cancer. The prepared nanoparticles are used for site-specific targeting to increase the efficacy of the drug and reduce the toxicity caused. The nanoparticles are been prepared using nanoprecipitation method. The prepared nanoparticles are been evaluated for particle size, zeta potential and for evaluating the particle shape using scanning electron microscopy.

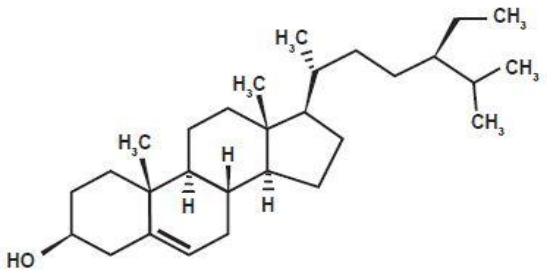

Figure 1: Structure of Beta-Sitosterol

Phytochemical Test for Steroids [7]

Salkowski test:

A few crystals of the drug were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution.

Observation**:** A reddish color was seen in the upper chloroform layer.

Liebermann Burchard test:

A few crystals were dissolved in the chloroform, add few drops of sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride.

Observation: Solution turned violet blue and finally green.

Liebermann's test:

A few crystals of the drug were added in acetic anhydride, heat and cool it followed by addition of concentrated sulfuric acid.

Observation: Blue color disappears.

Thin Layer Chromatography of Beta Sitosterol [8]

Preparation of Test Solution

10 mg of the drug is been dissolved in 10 ml of methanol to obtain a solution of 1000 µg/ml.

A small amount of test solution is been taken and a small band is applied to the TLC plate and is inserted in the TLC chamber for running the mobile phase. Later on, the plate is dried and is dipped in the vanillin-sulfuric acid reagent.

Preparation of Sample solution

10 mg of drug is been dissolved in 10 ml methanol to obtain the solution of 1000µg/ml.

A small amount of sample solution is been dissolved in 10 ml methanol. A small band is been applied on TLC plate and then inserted in the TLC chamber for running of the mobile phase. Later on, the plate is dried and dipped in the vanillin-sulfuric acid reagent.

Table 1: Mobile phases used for Test solution and Sample solution of TLC [9]

Mobile Phase Composition	Ratio of mobile phases	Retention factor
Toluene: Ethyl acetate: Glacial acetic acid	8: 2: 0.2	0.72
Benzene: methanol	9: 1	0.61
Ethyl acetate: n-hexane	4: 6	0.77
Toluene: Ethyl acetate	8: 2	0.52
Toluene: Methanol	9: 1	0.55

High-Performance Thin Layer Chromatography (HPTLC) [8]

Preparation of Sample solution:

10 mg of sample is dissolved in 5 ml of methanol, later on, make up the volume up to 10 ml to produce the solution of 1000 µg/ml.

Preparation of Test solution:

10 mg of the test sample is dissolved in 5 ml of methanol, later on, make up the volume up to 10 ml to produce the solution of 1000 µg/ml.

Solvents sued for Mobile phase:

As ethyl acetate and hexane show greater retention factor, this is been taken as the mobile phase for the drug.

Ethyl acetate: n-hexane (4: 6)

Preparation of TLC plates for the collection of spots: [7, 8, 9]

Silica gel H is been used as an adsorbent for a collection of spots in TLC, the TLC plates were prepared and heated for activation in an oven for 30 minutes at 110˚C. The preheated TLC plates were used for observation of spots after collection. A small amount of sample and test solution were spotted on TLC plate using micropipette of the CAMAG HPTLC and inserted TLC plate is allowed to dry and later on dipped in vanillin-sulfuric acid for detection of the spot. Retention factor is been calculated accordingly by using formula

Retention factor (R_f) = Distance traveled by solute / Distance traveled by a solvent.

The standard graph for Beta-Sitosterol

Preparation of Standard stock solution

Standard Beta Sitosterol 10 mg was accurately weighed and transferred to a 10 ml volumetric flask. It was dissolved properly and diluted up to mark with Phosphate buffer (pH-7.4) to obtain a concentration of 1mg/ml. This solution was used as a working standard solution. From this solution, appropriate dilutions are been made.

Chromatographic Scanning of Beta-Sitosterol

From the solution, the scanning is done and the scan was taken between the wavelength 200-800 nm which gave the highest peak at 550 nm and the same was selected maximum wavelength for Beta Sitosterol.

Preparation of standard plot for Beta-Sitosterol

From the standard solution series of dilution were made to get 1, 2, 3, 4 and 5 ppm solution using Phosphate buffer. A solution of 1, 2, 3, 4 and 5 ppm was prepared by pipetting out the required amount of solutions from stock solution and making up the volume up to 10 ml with phosphate buffer and area under the curve was measured by using High-performance thin layer chromatography.

Table 2: Calibration curve of Beta Sitosterol

Concentration (µg/ml)	Area under curve
1	2500
2	5500
3	8267.5
4	10782.2
5	14048.8

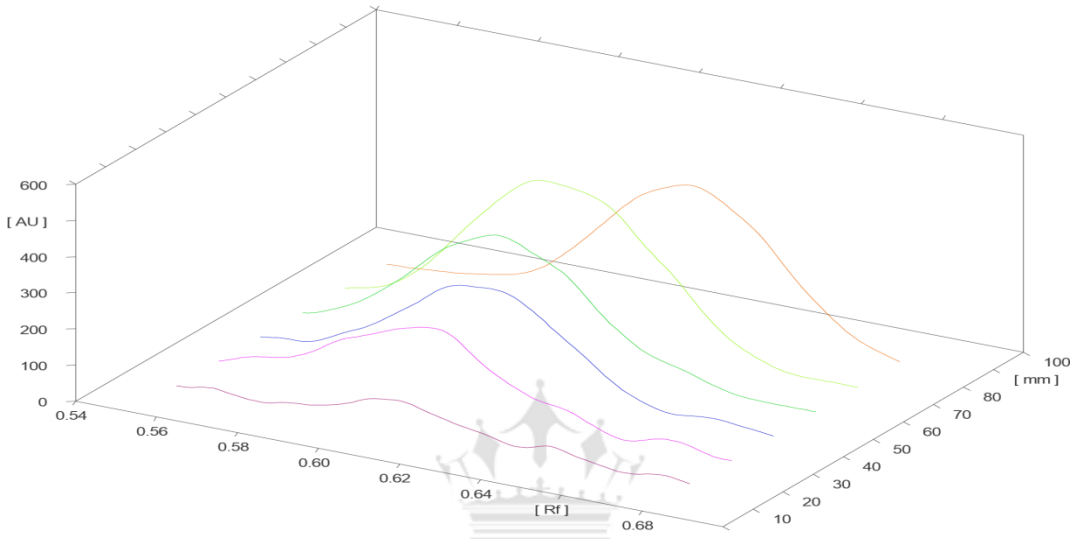

Figure 2: Chromatogram of Beta Sitosterol

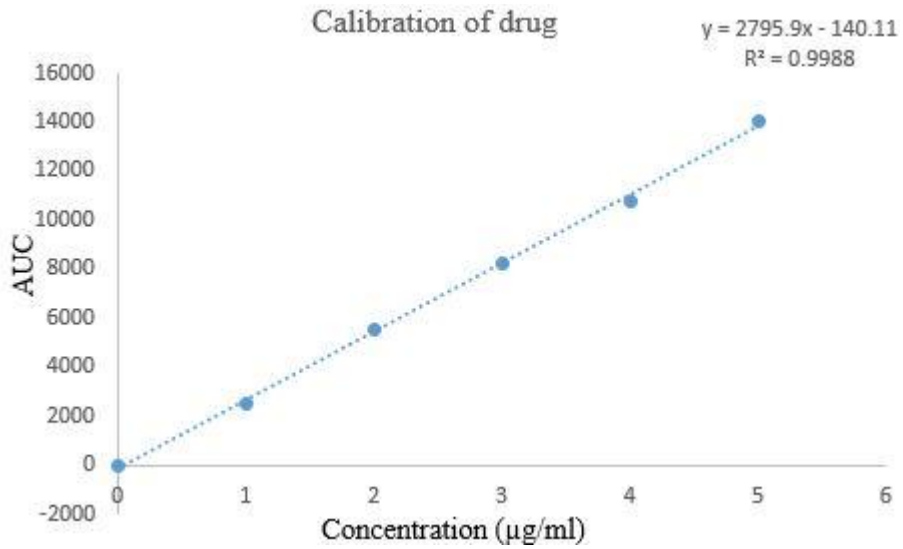

Figure 3: Calibration curve graph of drug

MATERIALS AND METHODS:

MATERIALS:

Beta Sitosterol was purchased from TCI chemicals (shah enterprises) Mumbai. Eudragit RL100 polymer was purchased from Evonik chemicals (India), Poloxamer 407 was a kind of gift sample from Glenmark chemicals. All the reagents were of analytical grade (AR grade).

Preparation of Nanoparticles

Drug-loaded eudragit nanoparticles are been prepared by nanoprecipitation method which is known as the modified version of the solvent evaporation method. The drug and polymer are taken in 1:10 ratio, i.e 250 mg of drug is taken with respect to 2500 mg of polymer, 2% of Poloxamer 407 is been used as an emulsifier or stabilizer in the preparation of nanoparticles. The polymer is been dissolved in acetone and is put on a magnetic stirrer for around 20-30 minutes at 350-450 rpm. The drug is also been dissolved in acetone and added dropwise in the polymeric mixture. And again stirred for 10-15 minutes. The stabilizer Poloxamer 407 is also been added dropwise in the drug and polymeric mixture and stirred on the magnetic stirrer for 2 hours at 450 rpm. The prepared nanoparticles are evaluated for appearance, homogeneity, flocculation, particle size, zeta potential, and particle shape.

Table 3: Optimization of the Nanoparticulate system.

Name of Ingredients	Formulation code		
	F1 (drug: polymer) (1:2.5)	F2 (drug: polymer) (1: 5)	F3 (drug: polymer) (1: 10)
Beta Sitosterol	250 mg	250 mg	250 mg
Eudragit RL 100	625 mg	1250 mg	2500 mg
Poloxamer 407	2%	2%	2%
Acetone	20 ml	20 ml	20 ml
Water	q. s	q. s	q. s

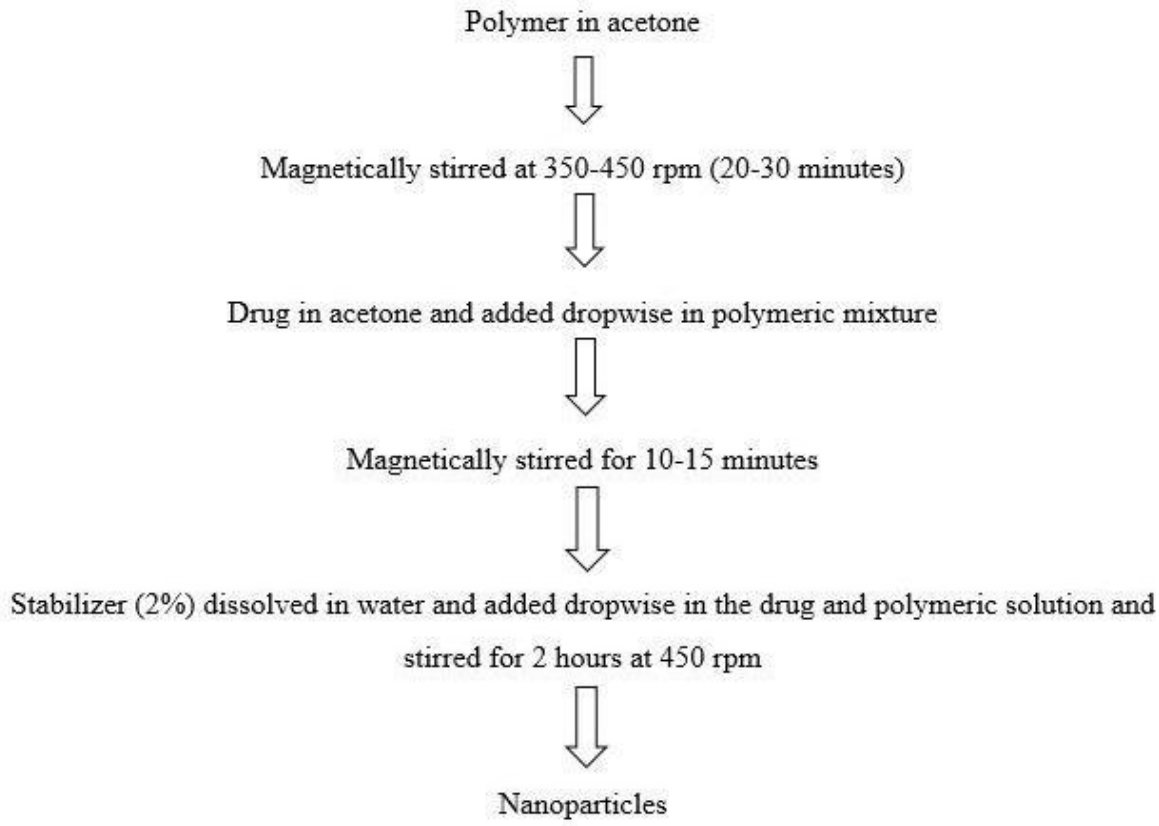

Figure 4: Flowchart for preparation of nanoparticles

Appearance: [1]

The nanoparticulate system was observed for color, homogeneity, and flocculation.

Particle size: [1, 9]

The particle size morphology and distribution are the most important parameters for the characterization of the nanoparticulate system. Particle size is been determined by using Malvern Zeta sizer following the principle of light scattering techniques. As particle size affects the drug release, smaller the particle size larger is the surface area showing greater action. All the 3 formulations were subjected for analysis of particle size analysis.

Zeta Potential: [1, 9]

The nature and intensity of the surface charge of nanoparticles are very important as it determines their interaction with the biological environment. The stability of the system can be determined using the Malvern Zeta potential. Zeta potential values can be negative or positive in order to achieve the stability of the system.

Drug Entrapment efficiency [1, 9]

Drug entrapment efficiency tells about the amount of the drug entrapped in the system. The nanoparticles were separated from the aqueous medium by centrifugation at 10,000 rpm for 30 minutes. Then the resulting supernatant solution was decanted and dispersed into phosphate buffer saline pH 7.4. Thus the procedure was repeated twice to remove unentrapped drug molecules completely. The amount of drug entrapped in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

Entrapment efficiency (%) = (Amount of entrapped drug (mg) / Total amount of added drug (mg)) ×100

***In-vitro* drug release [1, 9]**

A central reason for pursuing nanotechnology is to deliver drugs, hence understanding the manner and extent to which the drug molecules are released is important. The drug loading of the nanoparticles is generally defined as the amount of drug bound per mass of the polymer (usual moles of drug per mg polymer or mg drug per m polymer); it could also be given as the percentage relative to the polymer. The following methods for the determination of the *in-vitro* release have been used:

- Side by side diffusion cells with artificial or biological membranes
- Dialysis bag diffusion technique
- Reverse dialysis sac technique
- Ultracentrifugation
- Ultrafiltration (Centrifugal) technique.

RESULTS

Physical Appearance of Nanoparticulate system

Appearance, Homogeneity, and Flocculation are been studied. All the formulations are seen white in color, homogenous and no flocculation is been seen.

Table 4: Physical visualization of nanoparticulate system

Formulation code	Appearance	Homogeneity	Flocculation
F1	White	Homogenous	No
F2	White	Homogenous	No
F3	White	Homogenous	No

Particle size:

The particle size is an important parameter of the nanoparticulate system. The size is been determined using the zeta sizer and the particle size of F3 was found to be appropriate that is 300 nm.

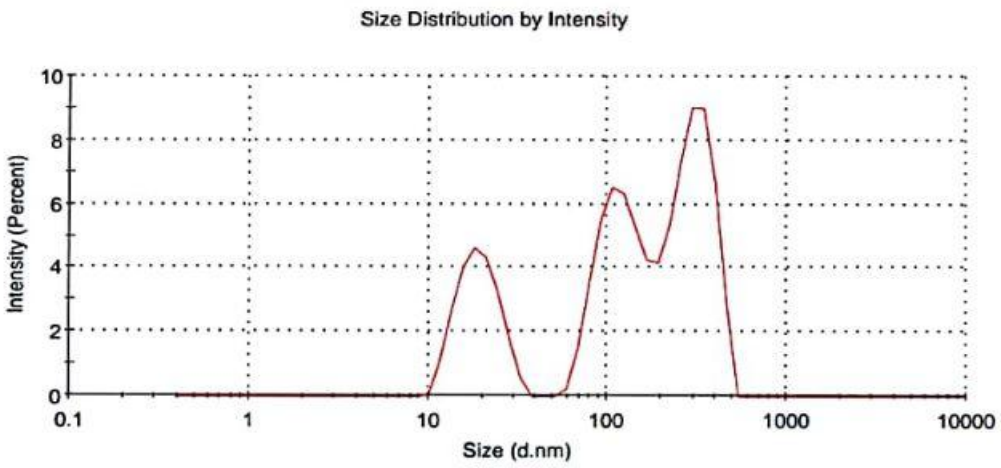

Figure 5: Particle size analysis of F3 formulation

Zeta Potential:

Zeta Potential is been determined using Malvern. It tells about the surface charge of the nanoparticles as well as their interaction with the biological environment. It also tells about the colloidal stability of the nanoparticles. The Zeta Potential of the system was found to be 6.91 mV.

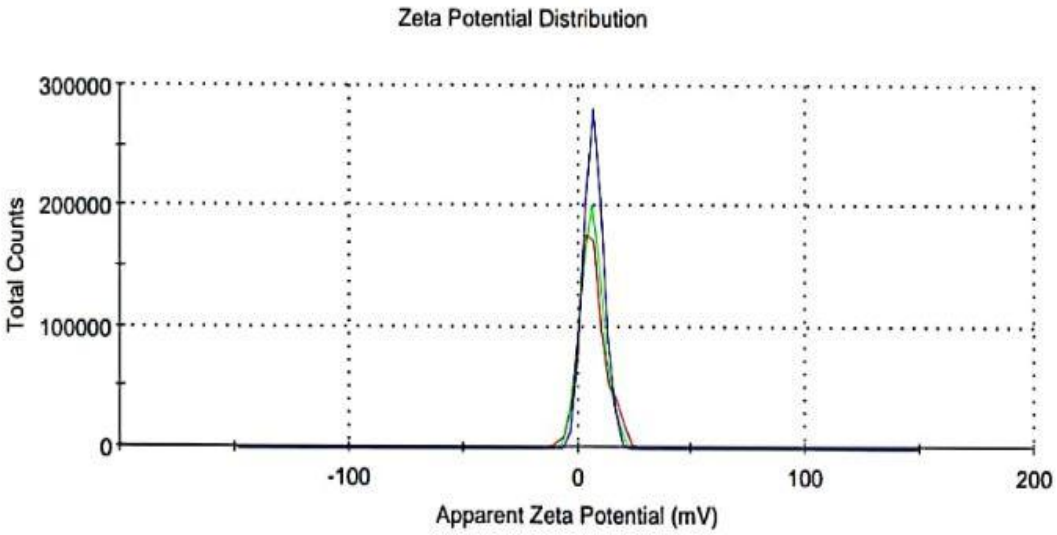

Figure 6: Zeta Potential

Scanning electron microscopy:

It determines the shape and size of nanoparticles, the nanoparticles were found to be spherical in shape or size 300 nm of the F3 formulation.

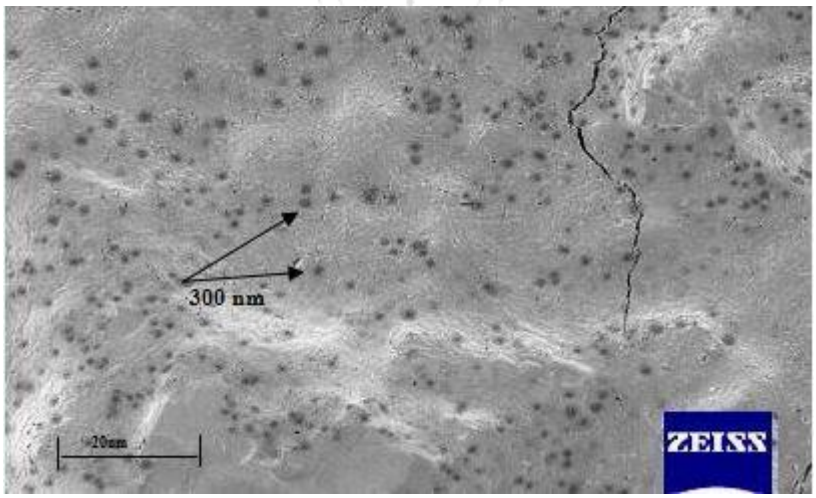

Figure 7: SEM image of the nanoparticulate system

Drug Entrapment Efficiency:

The drug entrapment efficiency of nanoparticles was found to be 80%

***In-vitro* drug release**

It's very essential to determine the extent of the drug release and in order to obtain such information, most release methods require that the drug and its delivery vehicle be separated. Drug loading capacity of the nanoparticles is defined as the amount of drug bound per mass of polymer or in another term, it is the moles of drug per mg polymer or mg drug per mg polymer or it could also be given as a percentage relative to the polymer.

Table 5: Drug Release of optimized formulation

Time (hrs)	% Cumulative Release
0	0.09
1	20.50
2	33.80
3	45.15
4	52.50
5	63.50
6	70.30
7	76.18
8	82.19

Drug Release Kinetics [15, 16]

Several drug release data on mathematical models can be obtained, hence drug release profile can be correlated with drug release kinetic models.

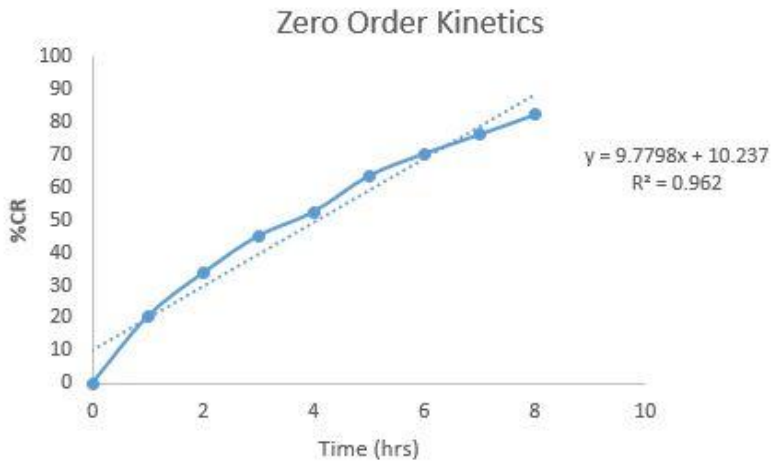

Figure 8: Zero order kinetics

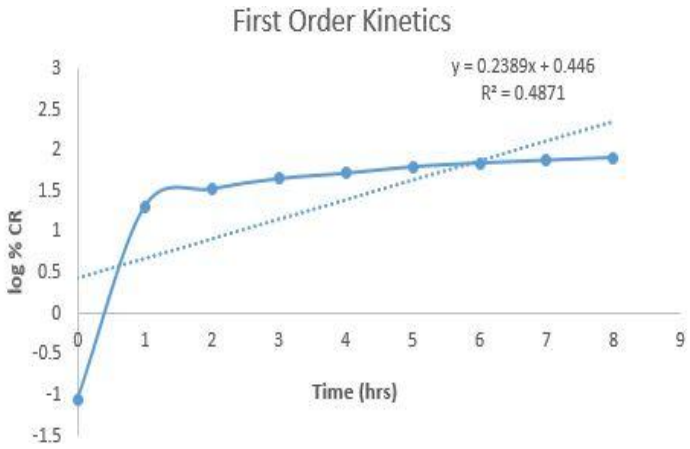

Figure 9: First order kinetics

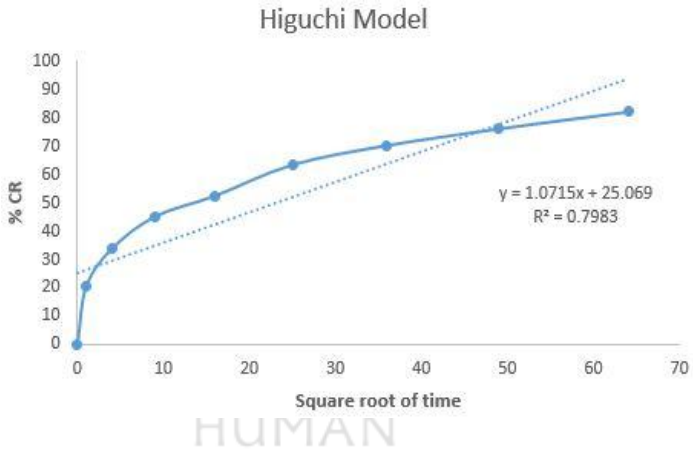

Figure 10: Higuchi Model

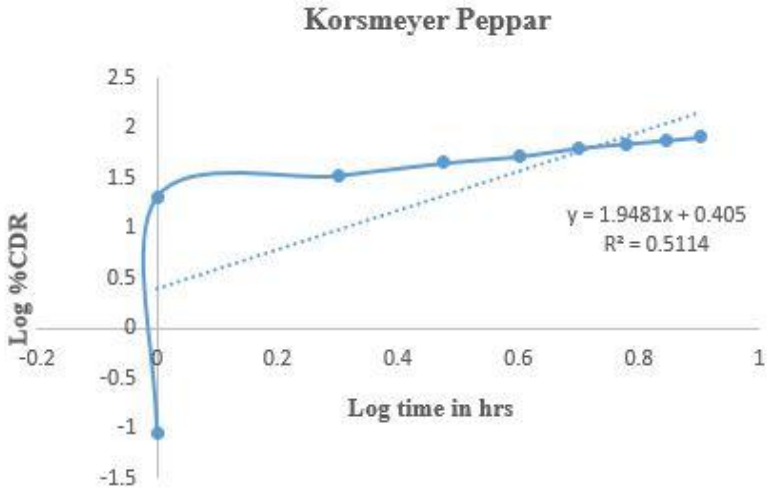

Figure 11: Korsmeyer Peppar

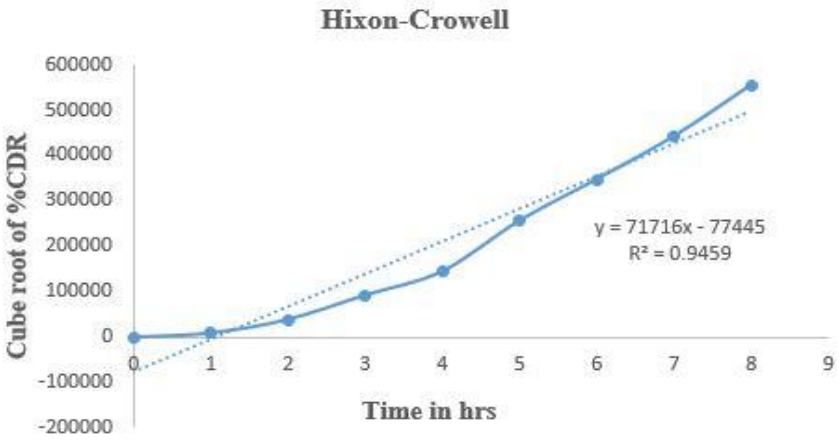

Figure 12: Hixon-Crowell

Zero-order kinetics shows the R2 value as 0.962 which is near to 1. Hence the formulation follows zero order kinetics.

Table 6: Drug release models

Models	Slope	Intercept	R^2
Zero order kinetics	9.7798	10.297	0.962
First order kinetics	0.2389	0.446	0.4871
Higuchi	1.0715	25.069	0.7983
Korsmeyer Peppar	1.9481	0.405	0.5114
Hixon-Crowell	71716	77445	0.9459

***In-vitro* antibacterial activity:**

The antibacterial activity of Beta Sitosterol against *E.coli, Bacillus subtilis, P. vulgaris* and *S. aureus* in the nanoparticulate system is been evaluated in which percentage inhibition was taken as a measure of the drug antibacterial activity. Thus the highest activity was observed with *P. vulgaris* and *S.aureus.*

Table 7: Percent inhibition of various bacterial species

Bacterial Species	% Inhibition
E.coli	58.90
P. vulgaris	69.85
S. aureus	72.89
B. subtilis	62.50

DISCUSSION:

Beta Sitosterol is a plant sterol obtained from plant Abelmoschus moschatus showing anti-cancer activity. This beta-Sitosterol is been entrapped in the nanoparticulate matrix to enhance the bioavailability of the drug along with its action thus reducing the side effects. The nanoparticles are been prepared by nanoprecipitation method. The required size that is 300 nm is been obtained of formulation 3 with entrapment efficiency of 80% and drug release 82.19% following the zero-order kinetics as the R^2 value is near to 1. Beta Sitosterol shows various activities along with anti-cancer such as anti-bacterial activity, anti-inflammatory, and anti-oxidant activity, out of which anti-bacterial activity is been proved.

CONCLUSION:

Novel drug delivery system has been achieved a lot of success in the medical applications to use very serious and life-threatening diseases. In this novel drug delivery system, a nanoparticulate system or the nanoparticles are been used as a targeted drug delivery system to target a specific disease-causing site to increase or greater the efficacy of drug with a less amount of dose resulting in less toxicity.

Nanoparticles are been helpful in providing the better action or efficacy of the drug due to its small particle size, as less the particle size more is the surface area and hence more is the action thus reducing the other toxic effects. It also becomes a solution for encapsulating the hydrophobic drug in a water-soluble gel base for long-term stability.

FUTURE PERSPECTIVE:

The anticancer activity of Beta-Sitosterol which is been used to treat various cancers like breast cancer, ovarian cancer, stomach cancer, lung cancer and leukemia will be done in future by using animals (animal study) and will be proved in future.

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