

## “Design and Assessment of Microspheres Containing Beta-Sitosterol: A Study on Antimicrobial Efficacy”

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### ABSTRACT

The present study was undertaken to formulate and characterize beta-sitosterol-loaded microspheres using the solvent evaporation technique. Preliminary trials confirmed the suitability of polymers for microsphere formation, and hydroxypropyl methylcellulose (HPMC) and ethyl cellulose were selected for further formulation studies. Scanning electron microscopy revealed that the prepared microspheres were spherical in shape with smooth and porous surface morphology. Particle size analysis using a Malvern zeta sizer confirmed that the microspheres were within the nanometer range, with particle sizes ranging from 181.4 to 823.8 nm. Zeta potential measurements indicated good stability of the formulated microspheres. High drug entrapment efficiency was observed for all formulations, demonstrating effective encapsulation of beta-sitosterol. The results indicated that formulation and process variables, particularly the drug-to-polymer ratio, significantly influenced particle size, entrapment efficiency, and drug release behavior. SEM analysis confirmed uniform drug distribution within the polymeric matrix without significant drug-polymer interaction. The developed beta-sitosterol microspheres show promise as a novel drug delivery system and may be further explored for incorporation into various dosage forms to enhance therapeutic efficacy.

**KEYWORDS:** Beta-sitosterol; Microspheres; Solvent evaporation method; HPMC; Ethyl cellulose; Drug entrapment efficiency; Controlled drug delivery

### I. INTRODUCTION

Topical drug delivery has gained significant attention in recent years due to its ability to provide localized therapeutic effects with reduced systemic side effects. Among various topical treatments, adapalene, a third-generation synthetic retinoid, is widely used in the

management of acne vulgaris owing to its comedolytic, anti-inflammatory, and keratolytic properties. However, its poor aqueous solubility, limited bioavailability, and potential for causing skin irritation restrict its therapeutic efficiency in conventional formulations (Gupta et al., 2022).

The term microsphere refers to free-flowing spherical particles made up of medicament and polymer matrix. They are composed of synthetic polymers or proteins which are biodegradable in nature and have a particle size of less than 200µm (Gurung and Kakar 2020). These are tiny, spherical particles and typically having dimensions between 1 to 1000 µm. Microspheres sometimes referred to as microparticles. There are two types of microspheres microcapsules and micromatrices. In micromatrices the entrapped material is distributed throughout the microsphere matrix. In microcapsules the entrapped material is enclosed by a discrete capsule wall (Lengyel et al., 2019).

Microspheres improve the drugs therapeutic efficacy and bioavailability, reduces toxicity and minimizes side effects. Microspheres can be prepared by various materials such as natural and synthetic materials. It plays a crucial role in enhancing the absorption of traditional medicines and microencapsulation is an alternative method to delay the release of medicine (Sivakumar et al., 2025). Because of smaller particle size it broadly dispersed throughout the GIT and improves drug absorption. The most convenient and preferable method is oral route of drug administration mainly frequent doses maintain steady plasma concentration and have low patient compliance (Alqahtaniet al., 2021).

Beta-sitosterol is a naturally occurring phytosterol widely found in plant sources such as nuts, seeds, fruits, and vegetables. Structurally similar to cholesterol, beta-sitosterol exhibits a wide range of pharmacological activities, including anti-inflammatory, antioxidant,

immunomodulatory, antihyperlipidemic, anticancer, and anti-diabetic effects (Saeidnia et al., 2014). It has also been extensively studied for its role in the management of benign prostatic hyperplasia, cardiovascular diseases, and inflammatory disorders. Despite its promising therapeutic potential, the clinical application of beta-sitosterol is significantly limited due to its poor aqueous solubility, low bioavailability, rapid metabolism, and inconsistent absorption following oral administration (Bin Sayeed et al., 2016).

Poor solubility is a major challenge associated with beta-sitosterol, as it belongs to the Biopharmaceutical Classification System (BCS) class II compounds, characterized by low solubility and high permeability (Christiansen, 2002). The low dissolution rate of beta-sitosterol in gastrointestinal fluids leads to reduced absorption and variable therapeutic response. Consequently, there is a growing need to develop advanced drug delivery systems that can enhance the solubility, stability, and controlled release of beta-sitosterol, thereby improving its bioavailability and therapeutic efficacy (Sangralet et al., 2025).

Therefore, the present study aims to design and assess microspheres containing beta-sitosterol with a particular focus on evaluating their antimicrobial efficacy. By developing a controlled drug delivery system, the study seeks to enhance the solubility, stability, and sustained release of beta-sitosterol, ultimately improving its antimicrobial potential. The successful development of beta-sitosterol-loaded microspheres may offer a promising alternative for the treatment of microbial infections and contribute to the advancement of natural product-based antimicrobial therapies.

## II. MATERIALS AND MEHODS

### 2.1 Chemicals

Ethanol, Methanol, DCM, DMSO, n-Octanol and Sodium hydroxide were obtained from Merck, a reputable supplier of analytical reagents. Rankem provided the Petroleum ether and Methanol. Sigma provided the HPMC, EC, and KBr. Beta-sitosterol were obtained from Carbanio.

### 2.1 Pre-formulation study

#### 2.1.1 Organoleptic Properties

Organoleptic properties of Beta-sitosterol were observed by visual observation. The organoleptic studies of Beta-sitosterol like general appearance like color, odor, state, etc. were performed.

### 2.1.2 Solubility study

Qualitative solubility of Beta-sitosterol in different solvents was determined according to USP NF, 2007. Approximately 1 mg of Beta-sitosterol was weighed and transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, DMOS ethanol, and water) (Jain and Verma 2020).

### 2.1.3 Melting Point

Melting point was analyzed by open Capillary method using Thiele's tube. (Chowk, M. I. 2020).

### 2.1.4 pH determination

pH was determined by Electrochemical method. Digital pH meter is used to determine the pH of Beta-sitosterol (Albalawi and Gadov 2024).

### 2.1.5 Partition coefficient determination

Partition coefficient (Log P) value is defined as ratio of unionized drug distributed between aqueous and organic phase. Oil-water partition coefficient gives the idea about drug's ability to cross the lipidic membrane. Lipophilic/hydrophilic balance is one of the most important contributing factors for optimum drug absorption and delivery. Due to lipidic nature of biological membrane, the amount of drug absorbed depends heavily on its lipophilicity. It is the unionized form of molecule that has better lipophilicity and hence it has received so much importance (S Bharateet al., 2016).

5 mg of drug was taken in separating funnel. The separating funnel was shaken for 2 hrs in a wrist action shaker for equilibration. Two phases were separated and the amount of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phase's was calculated by using formula:

$$\text{Partition Coefficient} = \frac{\text{Concentration of drug in oil phase}}{\text{Concentration of drug in aqueous phase}}$$

### 2.1.6 Determination of Lambda max and calibration curve

#### • Lambda ( $\lambda$ ) max

A stock standard solution containing 1 mg/mL of Beta-sitosterol was prepared in methanol. Working standard solution equivalent to 100  $\mu$ g/mL of Beta-sitosterol was prepared by appropriate dilution of stock solution with the same solvent. The solution was scanned in the range of 200 – 800 nm UV spectrum using shimadzu UV-visible spectrophotometer -1700 (Kumbhar and Salunkhe 2013).

• **Standard calibration curve of Beta-sitosterol**

**Preparation of calibration curve**

The prepared stock solution was further diluted with solvent to get working standard solution of 10, 20, 30, 40, 50, and 60 µg/ml of Beta-sitosterol to construct Beer's law plot for the pure drug, the absorbance was measured at λ max at 212.0 nm, against solvent as blank. The standard graph was plotted by taking concentration of drug on X-axis and absorbance on Y-axis in the concentration range of 10-60µg/ml. (Behera et al., 2012).

**2.1.7 Fourier transmission Infra-Red Spectroscopy**

FT-IR spectrum of Drug was recorded over the range of 4000 to 400 cm<sup>-1</sup> by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of Drug and 100 mg of spectroscopic grade KBr which has been dried

using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm<sup>-1</sup> region (Chowk, M. I. 2020)

**2.2 Formulation of microspheres by Solvent Evaporation method**

Microspheres containing Beta-sitosteroldrug as a core material were prepared by Solvent Evaporation method. Drug (Beta-sitosterol), HPMC and EC were dissolved in a mixture of ethanol and dichloromethane (1:1) at room temperature (As in table 7). This was poured into 250 mL water containing 0.01% Tween-80 maintained at a temperature of 30–40 °C and subsequently stirred at 300 rpm agitation speed for 45 minutes to allow the volatile solvent to evaporate. The microspheres formed were filtered, washed with water and dried in oven at 37°C (Fartyal et al., 2011).

**Table 1: Composition of microsphere formulation**

Formulations (Code)	Polymer ratio HPMC (mg)	Polymer ratio Ethyl cellulose (mg)	Twee n-80 (%)	Drug (Beta-sitosterol)	Temper ature °C	Solvent ratio(1:1) ethanol/DCM
F1	300	100	0.01%	100	30-40°C	5ml:5ml
F2	250	150	0.01%	100	30-40°C	5ml:5ml
F3	200	200	0.01%	100	30-40°C	5ml:5ml
F4	150	250	0.01%	100	30-40°C	5ml:5ml
F5	100	300	0.01%	100	30-40°C	5ml:5ml

**2.3 Evaluation parameter of drug loaded microsphere**

**2.3.1 Particle size**

The particle size is one of the most important parameter for the characterization of microspheres. The size of microspheres was measured using Malvern Zeta sizer (Malvern Instruments) (Singh and Vingkar 2008).

**2.3.2 Zeta potential**

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field and the particle charge (Đordević et al., 2015).

**2.3.3 Quantitative analysis (Entrapment Efficiency)**

%Entrapment efficiency was determined by indirect estimation. Drug -loaded microspheres were centrifuged at 15,000 rpm for 30 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant

solution using UV spectrophotometer. The peak area was determined and amount of free drug is determined by extrapolating the calibration curve. And drug entrapment calculated by using below equation. The entrapment efficiency data is documented in Table 16 (Balla and Goli 2020).

**Entrapment efficiency % = Total drug conc. - Supernatant drug conc. / total drug conc.\*100**

**2.3.4 Scanning Electron Microscopic (SEM)**

The electron beam from a scanning electron microscope was used to attain the morphological features of the drug loaded microspheres were coated with a thin layer (2–20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pretreated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further

processed based on Rutherford and Kramer’s Law for acquiring the images of surface topography (Anweret al., 2019).

#### 2.4 In-vitro drug release study

The in-vitro drug release study of drug loaded Microsphere formulation was studied by dialysis bag diffusion method. Microspheres were dispersed into dialysis bag and the dialysis bag was then kept in a beaker containing 100 ml of pH 7.4 phosphate buffer. The beaker was placed over a magnetic stirrer and the temperature of the assembly was maintained at  $37 \pm 2$  °C throughout the experiment. During the experiment rpm was maintained at 100 rpm. Samples (2 ml) were withdrawn at a definite time intervals and replaced with equal amounts of fresh pH 7.4 phosphate buffers. After suitable dilutions the samples were analyzed using UV–Visible spectrophotometer at 352 nm. To analyze the in vitro drug release data various kinetic models were used to describe the release kinetics.

#### 2.5 Antibacterial activity of Microsphere by Well diffusion assay

- Preparation of Nutrient Agar Media**  
 28 g of Nutrient Media was dissolved in 1litre of distilled water. pH of media was checked before sterilization. Media was sterilized in autoclave at 121°C at 15 lbs pressure for 15 minutes. Nutrient media was poured into plates and placed in the laminar air flow until the agar was get solidified.
- Well Diffusion Assay**  
 The bacterial suspension of E. coli was standardized to  $10^8$  CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculums from the broth (containing  $10^8$  CFU/ml) was taken with a micropipette and then transferred to fresh and

sterile solidified Agar Media Plate. The agar plate was inoculated by spreading the inoculums with a sterile spreader, over the entire sterile agar surface. Three wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer. The wells were then formed for the inoculation of the microsphere, Microsphere and drug (1mg/ml) solution. 100 µl of the sample was loaded. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37° C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. Zones were measured to a nearest millimeter using a ruler, which was held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black, non-reflecting background. The diameters of the zone of complete inhibition (as judge by unaided eye) were measured, including the diameter of the well (Sunanda and Babu 2025).

#### 2.6 Stability studies

The drug loaded Microsphere formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing ( $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $60 \pm 5\%$  RH) and ( $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and  $70 \pm 5\%$  RH) for 3 months. The formulation were checked for evaluation parameter particle size, entrapment efficiency and in-vitro drug release studies at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in evaluation parameter particle size, entrapment efficiency and in-vitro drug release studies (Li et al., 2016).

### III. RESULT AND DISCUSSION

#### 3.1 Organoleptic properties

Table 2: Organoleptic properties of Beta-sitosterol

Drug	Organoleptic properties	Observation
Beta-sitosterol	Color	white
	Odor	Characteristic
	Appearance	waxy Powder
	State	Solid Powder

### 3.2 Melting point study

**Table 3: Melting point study of Beta-sitosterol**

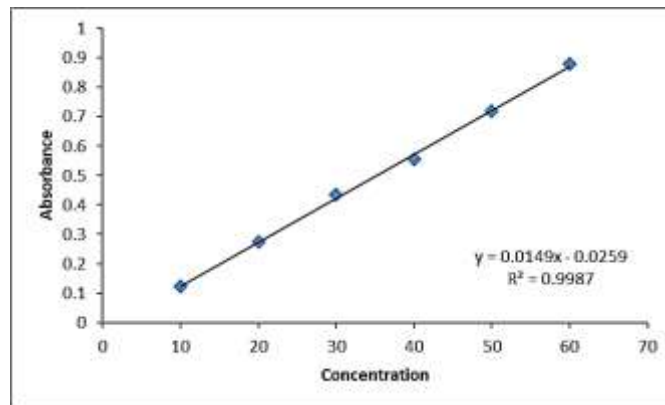
Drugs	Observed	Reference
Beta-sitosterol	137°C	135°C-137°C

### 3.3 Determination of Partition coefficient

**Table 4: Partition coefficient:**

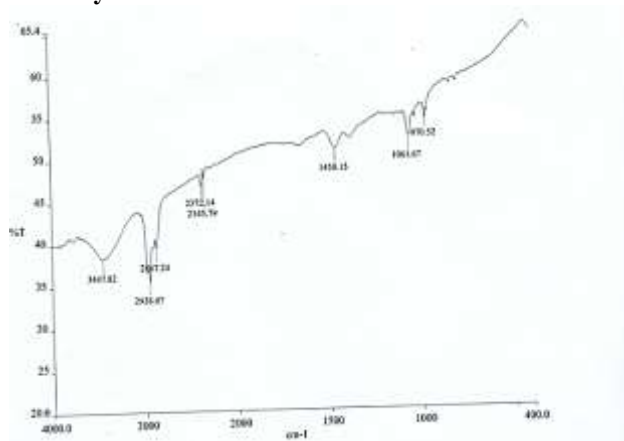
Drug	Solvent	Partition coefficient
Beta-sitosterol	n-Octanol: water	0.65

### 3.4 Calibration curve



**Graph 1: Calibration curve of Beta-sitosterol**

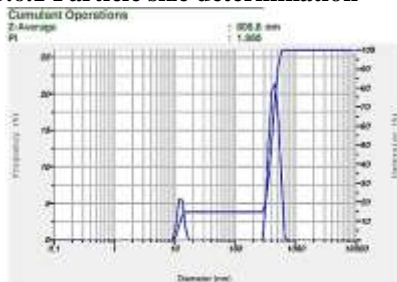
### 3.5 Functional group identified by FTIR



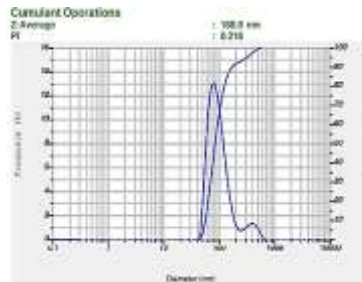
**Graph 2: FTIR study**

### 3.6 Evaluation parameter of microspheres formulation

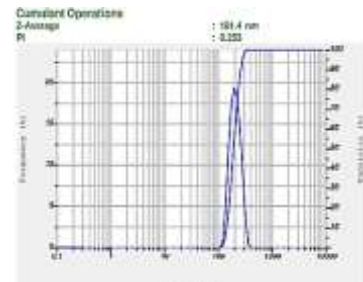
#### 3.6.1 Particle size determination



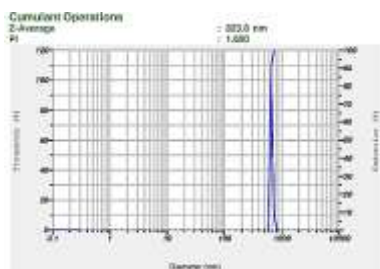
**Group 3: Particle size (F1)**



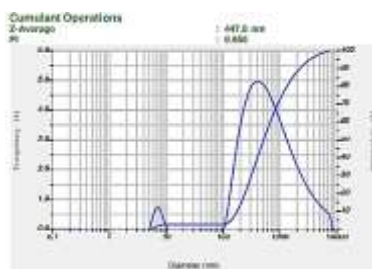
**Group 4: Particle size (F2)**



**Group 5: Particle size (F3)**



Group 6: Particle size (F4)



Group 7: Particle size (F5)

### 3.6.2 Zeta potential determination

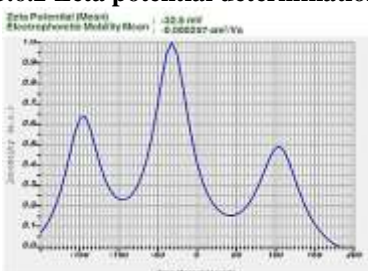


Figure 1: Zeta potential (F1)

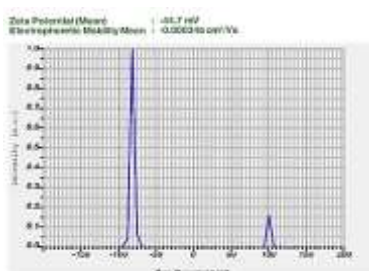


Figure 2: Zeta potential (F2)

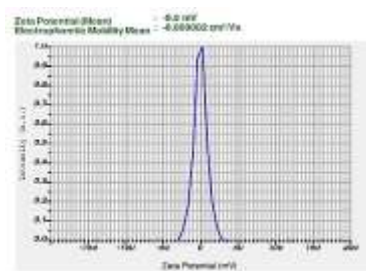


Figure 3: Zeta potential (F3)

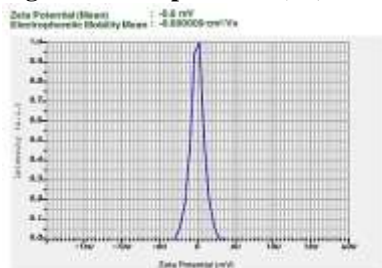


Figure 4: Zeta potential (F4)

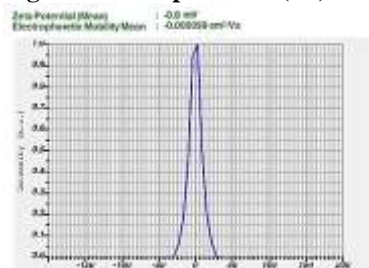


Figure 5: Zeta potential (F5)

Table 6: Result of Particle size, Zeta potential and Entrapment efficacy of all formulations

Formulation	Zeta potential	Particle size (nm)	PI Value	Entrapment efficacy (%)
Microsphere (F1)	-32.5 mV	808.8	1.860	75.99
Microsphere (F2)	-44.7 mV	188.0	0.218	77.87
Microsphere (F3)	-0.5 mV	181.4	0.253	<b>90.03</b>
Microsphere (F4)	-0.6 mV	823.8	1.650	84.98
Microsphere (F5)	-0.8 mV	447.8	0.655	87.05

### 3.6.3 Scanning electron microscopy characterization of F3 formulation

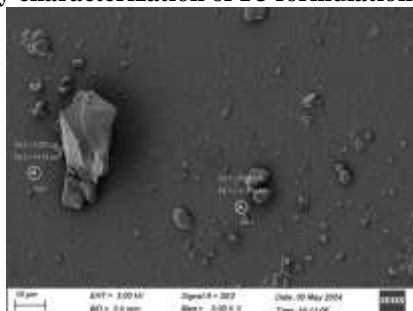


Figure 1: SEM

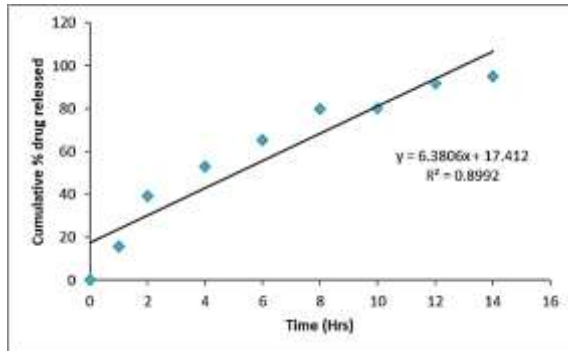
3.6.4 In- vitro drug release kinetics study of F3 formulation

Table 7: Release kinetics study of F3 formulation

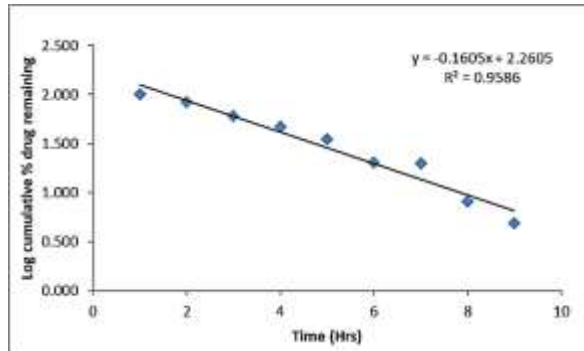
Time (hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	log Cumu % drug released
0	0	100	0.000	2.000	0.000	0.000
1	15.82	84.18	1.000	1.925	0.000	1.199
2	39.39	60.61	1.414	1.783	0.301	1.595
4	53.08	46.92	2.000	1.671	0.602	1.725
6	65.18	34.82	2.449	1.542	0.778	1.814
8	79.78	20.22	2.828	1.306	0.903	1.902
10	80.16	19.84	3.162	1.298	1.000	1.904
12	91.86	8.14	3.464	0.911	1.079	1.963
14	95.13	4.87	3.742	0.688	1.146	1.978

Table 8: Correlation value (R<sup>2</sup> value)

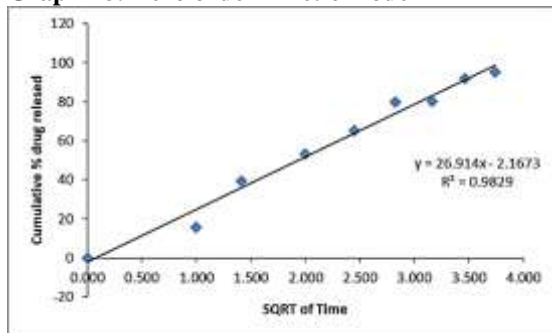
Formulation	Model	Kinetic parameter values
Microsphere	Zero Order	R <sup>2</sup> = 0.899
	First Order	R <sup>2</sup> = 0.958
	Higuchi	R <sup>2</sup> = 0.982
	Korsmeyerpeppas	R <sup>2</sup> = 0.664



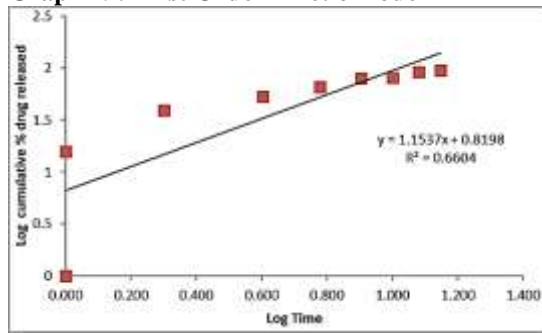
Graph 18: Zero order kinetic model



Graph 19: First Order kinetic model

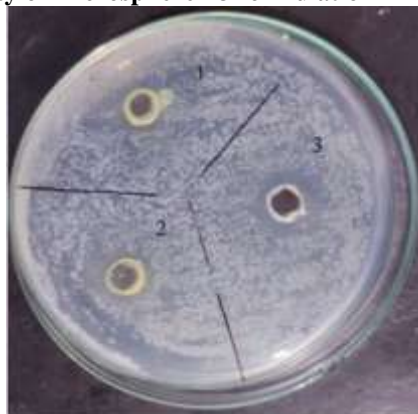


Graph 20: Higuchi model



Graph 21: Korsmeyerpeppas

### 3.7 Results of antimicrobial activity of microsphere F3 formulation



**Table 9: Antimicrobial activity of Formulation against E.coli**

Sample Name	Zone of Inhibition (mm)
Placebo	4 mm
Beta-cyclodextrin	11 mm
Formulation	15mm

### 3.8 Stability study

**Table 10: Stability Study of Microsphere (F3) formulation**

Time (Days)	25 <sup>0</sup> C±2 <sup>0</sup> C and 60 ± 5% RH				40 <sup>0</sup> C±2 <sup>0</sup> C and 70 ± 5% RH			
	Appearance	Particle size nm	Zeta potential mV	EE (%)	Appearance	Particle size nm	Zeta potential mV	EE (%)
0	Solid Powder	181.4 nm	-0.5 mV	90.03	Solid Powder	181.4 nm	-0.5 mV	90.03
30	Solid Powder	181.0 nm	-0.6 mV	90.01	Solid Powder	181.1 nm	-0.7 mV	90.00
45	Solid Powder	181.2 nm	-0.8mV	90.03	Solid Powder	181.0 nm	-0.8 mV	90.06
60	Solid Powder	179.9 nm	-0.9 mV	90.00	Solid Powder	181.4 nm	-1.0 mV	90.05
90	Solid Powder	180.2 nm	-1.0 mV	90.04	Solid Powder	181.4 nm	-1.1 mV	90.06

### Discussion

Beta-sitosterol was characterized for its organoleptic and physicochemical properties and found to comply with I.P. specifications. The drug appeared as a white, waxy solid with a characteristic odor and showed good solubility in methanol, ethanol, and DMSO, while being insoluble in water. The melting point (137 °C), partition coefficient (0.65), and λ<sub>max</sub> (212 nm) confirmed its identity and lipophilic nature. UV spectrophotometric analysis demonstrated good linearity over the concentration range of 10–60 µg/mL with a correlation coefficient of 0.987.

Beta-sitosterol-loaded microspheres were successfully formulated, and particle size analysis

confirmed sizes ranging from 181.4 to 823.8 nm. Zeta potential values (–0.5 to –44.7 mV) indicated good colloidal stability. High drug entrapment efficiency (75.99–90.03%) was achieved, with formulation F3 showing the highest entrapment due to optimized polymer concentration. SEM analysis revealed spherical microspheres with smooth surface morphology, confirming successful microsphere formation. In vitro drug release studies showed sustained release behavior. Kinetic modeling indicated that formulation F6 followed zero-order release kinetics (R<sup>2</sup> = 0.8992), suggesting concentration-independent drug release. Stability studies conducted under accelerated conditions demonstrated that the microsphere

formulations remained physically and chemically stable over three months, with no significant changes in appearance, particle size, zeta potential, or entrapment efficiency.

Overall, the results confirm that polymer concentration and formulation variables significantly influenced microsphere characteristics and drug release behavior, demonstrating the potential of beta-sitosterol-loaded microspheres as a stable and effective drug delivery system.

#### IV. CONCLUSION

Beta-sitosterol-loaded microspheres were successfully formulated using the solvent evaporation method with HPMC and ethyl cellulose. SEM analysis confirmed spherical microspheres with a smooth and porous surface morphology. Particle size analysis showed nanoscale dimensions, and zeta potential values indicated good stability. All formulations exhibited high drug entrapment efficiency. The results demonstrated that formulation and process variables significantly influenced particle size, drug release, and entrapment efficiency. Overall, the developed microspheres show potential as a novel drug delivery system for beta-sitosterol and can be further explored for various pharmaceutical dosage forms.

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