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Quality by Design (QBD) Assisted Formulation and Development of Microsphere by Design of Expert (DOE) Approach

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Abstract
This study presents the formulation and development of microspheres containing beta-sitosterol. The pH of beta-sitosterol was found to be 7.2, which is within the acceptable range specified for the drug. Its melting point was observed between 160°C and 174°C, aligning with the expected pharmaceutical specifications. A double-beam UV-Visible spectrophotometer (Shimadzu-1700) was used to determine the compound's absorption maxima (λmax), which was found to be 203.0 nm. Eudragit S 100 (polymer) and Tween 80 (surfactant) were selected for further study. Scanning electron microscopy (SEM) at 3,000x magnification revealed that the microspheres were spherical with a smooth surface and a porous structure. Particle size and zeta potential were measured using a Malvern Zetasizer. The analysis confirmed the nanoparticles were in the nanoscale range, with an average particle size of 430.7 nm. The zeta potential of -25.1 mV indicated good stability of the microsphere formulation. The optimized formulation also showed high drug entrapment efficiency, reaching 88.93%. These findings suggest that the Design of Experiments (DOE) approach, guided by the principles of Quality by Design (QbD), is effective for optimizing formulation and process parameters in the development of beta-sitosterol-loaded microspheres.

Keywords: Beta Sitosterol, Microsphere, Eudragit S 100, Tween 80, Design of Expert (DOE)

1. Introduction

Quality by Design: Quality by Design (QbD) is a methodical approach to development that starts with predetermined goals and stresses process control and product and process understanding. A methodical and controlled approach to identifying the connections between the variables influencing a process and its output is experimental design. The term "Design of Experiments" (DoE) is another name for it. In other words, the latter is the method of establishing mathematical connections between the inputs and

outputs of a process in order to attain process knowledge. [1]
Microspheres are small, spherical particles, typically ranging from 1 to 1000 micrometers in size that can be used as a drug delivery system. They are versatile and offer advantages like controlled and targeted drug release, improved bioavailability, and reduced side effects compared to conventional dosage forms. Microspheres can be prepared using various methods, including emulsification, spray drying, and polymerization, and are often composed of biodegradable or biocompatible polymers. β-sitosterol is a promising natural substance for the management of cholesterol and inflammation. The pharmacokinetic profile of β-sitosterol has also been extensively investigated. [2]In the present research, the modern DoE, custom design has been constructed simultaneously to screen and optimize beta sitosterol-loaded microspheres. This approach collects comprehensive data on multiple variables and their interactions in a single step, providing a thorough understanding of the selected variables.The improved predictive capability of custom designs creates accurate models, identifying and mitigating risks early in the development process [3]

2. Material and Methods

a. Chemicals

Chloroform, DMSO, and Petroleum ether were obtained from Renkem, a reputable supplier of analytical reagents. Shiva Chemical Co. provided the Ethyl acetate, and Lab Reagent LR Grade provided the Ethanol. Astron Chemicals (India) rovided the Methanol, while LabChem Industries supplied the conce Methyl Paraben. Himedia supplied the Tween 80, and SRL AR Grade provided the KBr. Chemical Corporation Pvt Ltd obtained from Sodiumcarboxy methylcellulose. Evonik Industries provided the Eudragit S100whileSigma-Aldrich supplied the Beta-sitosterol.

b. Pre-formulation study of Beta-Sitosterol

The first phase in the typical development of kinds of dosing for any medicinal ingredient is pre-formulation testing. The study of the chemical and physical characteristics of a pharmacological compound, either by itself or in combination with excipients, is known as pre-formulation. **[4]**

i. Organolepticevaluation

Organoleptic properties were observed by visual observation. It is the initial evaluation during Pre-formulation studies which assess the color, odor and looks of the substance.

ii. Solubility study

The solubility study was measured indifferent polar and non-polar solvent.**[5]**

iii. Determination of pH

pH was measured by digital pH meter. They are help to identify the acidity or basicity of the sample. **[6]**

iv. Determination of Melting point

With the help regarding the melting point, we can get the pristine nature of the sample. The point of melting was determined by use of open Capillary method using Thiele's tube by filling the drug samples on one closed side of the capillary tube. Liquid paraffin oil was completed in the Thieles tube and positioned within the contact of flame. **[7]**

v. Determination of Maximum Wave length (λmax)

1. **Preparation of Beta-sitosterol standard stock solution in methanol**

Standard solution of Beta-sitosterol was prepared by dissolving accurately weighed 10 mg of Beta-sitosterol in a 10 ml volumetric flask with 5 ml of methanol solvent. The volume was made up to 10 ml with methanol to obtain a stock solution of 1000 µg/ml. 1ml of this stock solution was taken and then diluted up to 10 ml using respective solvent (methanol) toacquire a solution that has a concentration 100 µg/ml which is standard stock solution.

2. Lambda max

A stock solution A10 ml volumetric flask was filled with 2 ml of the sample, and the volume was made up to mark with methanol to prepare a concentration of 20 µg/ml. The standard working solution for the drug was examined between 200 and 400 nm in the UV spectrum in normal mode, using distilled water as blank. Then obtained peaks were noted and the absorption maxima were determined. [8]

2.2.5.3LinearityandCalibrationCurve

From the working standard solution of 100 µg/mL, range of dilutions that were made was 5, 10, 15, 20, 25, 30, and 35 µg/mL. After precisely transferring the beta-sitosterol working standard stock solution into a series of 5 mL calibrated flasks, the volume was adjusted with methanol. At Beta-sitosterol 294.00 nm, the absorbance of the resultant solutions was measured in comparison to a blank of distilled water. A seven-point the calibration curve was produced for beta-sitosterol concentrations ranging from 5 to 35 µg/ml. [9]

vi. Functional group identified by FTIR

The microspheres' FTIR spectra were recorded by KBr press pellet technique and scanning from 400 – 4000cm-1. The KBr disc was made with 1 mg of beta-sitosterol in 100 mg of spectro scopic grade KBr which has been dried using IRl amp. The medication and KB rwer combined, and the disc was formed by applying hydraulic pressure. This disc was placed in FT-IR chamber [10]

c. Formulation of Microsphere

Microsphere formulations using Eudragit S100 were made using the emulsion solvent evaporation process as a carrier polymer. In 20 milliliters of chloroform, the desired amount of Eudragit S100 polymer was dissolved to create a homogenous polymer solution. The resulting mixture was then added to 250 ml of sodium CMC (0.5%) aqueous mucilage containing 0.1 to 0.5% v/v tween 80, while stirring at 1000 rpm for emulsification. Tween 80 was employed as a dispersion agent, sodium CMC aqueous mucilage served as a microencapsulating vehicle, and chloroform served as the polymer solvent. To obtain free- flowing microspheres, the microspheres were collected using vacuum filtration, repeatedly cleaned with petroleum the rand distilled water, and the nall owed to dryat room temperature for a full day. [11]

Table1: Composition of microsphere formulation

S. No	Formulation Code	Polymer Eudragit S100 (mg)	Surfactant Tween 80 (%)	Sodium CMC (%)	Drug (mg)	Chloroform	Water (ml)	Stirring time
1	MS1	300	0.3	0.5	30	20	250	1
2	MS2	300	0.3	0.5	30	20	250	3
3	MS3	300	0.5	0.5	30	20	250	2
4	MS4	175	0.1	0.5	30	20	250	3
5	MS5	50	0.1	0.5	30	20	250	2

6	MS6	175	0.1	0.5	30	20	250	1
7	MS7	50	0.3	0.5	30	20	250	3
8	MS8	50	0.3	0.5	30	20	250	1
9	MS9	175	0.5	0.5	30	20	250	3
10	MS 10	50	0.5	0.5	30	20	250	2
11	MS 11	300	0.1	0.5	30	20	250	2
12	MS 12	175	0.5	0.5	30	20	250	1

d. Design of experiment

Using Design of Experiment (Version 12.0.1.0) software, the experiment was designed for the microsphere formulation. In this instance, the second-order polynomial model represented the quadratic response surfaces.

i. Independent and Dependent variables

Table2: Independent and Dependent variables

Independent variables	Dependent variables
(X1)Polymer EudragitS100(mg)	(Y1)Particle size (nm)
(X2)Surfactant Tween80(mg)	(Y2)EE(%)
(X3)Stirring time(Min.)	

ii. Values of variables

Table 3: Values of variables

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Polymer	mg	Numeric	50.00	300.00	-1↔ 50.00	+1↔ 300.00	175.00	106.60
B	Surfactant	%	Numeric	0.1000	0.5000	-1↔ 0.10	+1↔ 0.50	0.3000	0.1706
C	Stirring time	hrs.	Numeric	1.0000	3.00	-1↔ 1.00	+1↔ 3.00	2.00	0.8528

e. Evaluation parameter of Drug loaded microsphere

i. **Particle size:** One of the most crucial parameters for characterizing microspheres is their particle size and zeta potential. The Malvern Zeta sizer (Malvern Instruments) was employed to gauge the microspheres' dimensions.

ii. **Scanning Electron Microscopic (SEM):** A scanning electron microscopes electron beam was utilized to acquire the morphological features of the drug-loaded microspheres. Under vacuum, a sputter coater applied a thin layer (2–20 nm) of metal (such as platinum, palladium, or gold).

iii. **Entrapment efficiency:** Indirect estimation was employed to determine the entrapment efficiency percentage. The REMIUltra Centrifuge was accustomed to centrifuge drug-loaded microspheres for 30 min at 15,000 rpm. Using a UV spectrophotometer, identify the medication that was entrapped in the supernatant solution.

Entrapment efficiency%=Total drug conc. –Supernatant drug conc./total drug conc. ×100

iv. ***In-vitro* drug release**

The dialysis bag diffusion method was employed to look into the drug release in vitro of microsphere-loaded compositions. A dialysis bag was filled with the

microsphere-loaded formulation and then put in a beaker containing 100 ml of phosphate buffer with a pH of 7.4. The beaker was placed over a magnetic stirrer to maintain the assembly's temperature at 37 ± 2 °C during the experiment. During the trial, the speed remained fixed at 100 rpm. At certain intervals, samples (2 ml) were taken out and swapped out with equal volumes of brand-new pH 7.4 phosphate buffers. A UV-visible spectrophotometer was used to analyze the samples at 301.0 nm after the proper dilutions. Several kinetic models were employed to characterize the release kinetics in order to interpret the in vitro drug release data.

f. Anti-Microbial Activity of Microsphere by Well diffusion assay

Well Diffusion Assay: In this method, 100µl bacterial suspension of *E. coli* was spread uniformly over the agar plates using sterile glass rod spreaders, to get uniform distribution of microbes. Thus, a sterile cork borer was used to aseptically create wells of 510 mm on the agar medium. Desired amount of material was aseptically filled into the well. Later the plates were positioned at room temperature for a time period to allowd if fusion of mater ialintoth agar. The plates were then incubated for particular period and the diameter of inhibition zone was recorded. [12]

g. Stability studies

A drug's stability has been described as the capability of a specific formulation, in an explicit container, to persist within its chemical, physical, toxicological, and therapeutic specifications. The purpose of stability testing is to make available the proof regarding how a product's quality drug ingredient or drug final product differs with duration under the effect of diverse environmental aspects such as humidity, temperature and light the suggested retest periods, storage conditions, and shelf life to be established. It was completed in air tight containers and later stored under the specified conditions for a time as mentioned by ICH guidelines for accelerated studies. [13]

3. Result and Discussion

a. Pre-formulation study of Beta-Sitosterol

Pre-formulation can be described as a study of the chemical and physical properties of a medication substance alone or when it is combined with excipients". Pre-formulation study aims to generate information that is beneficial for the formulation development personallyby developingstable andbioavailable dosage forms. The followingpre-formulationstudieswere performed for Beta-Sitosterol substances**.**

Table 4: Organoleptice valuation of Beta-Sitosterol

	Physical Observation No parameter	**Observation No**
Color	White	
Color	Characteristic	
State	Crystalline	
Appearance		Solid powder

Table 5: Solubility study of Beta-Sitosterol

Drug	**Solvents**	**Observation/Inference**
	Water	insoluble
	Ethanol	Soluble
	Methanol	Least Soluble
Beta- Sitosterol	Acetone	soluble
	DMSO	Soluble

	Ethyl acetate	Highly Soluble

Table 6: pH and melting point of beta-sitosterol

S.No.	Drug	Observed(pH)	MeltingPoint
1	Beta-Sitosterol	7.2	139^{0}C

b. Determination of λ max by UV spectroscopy

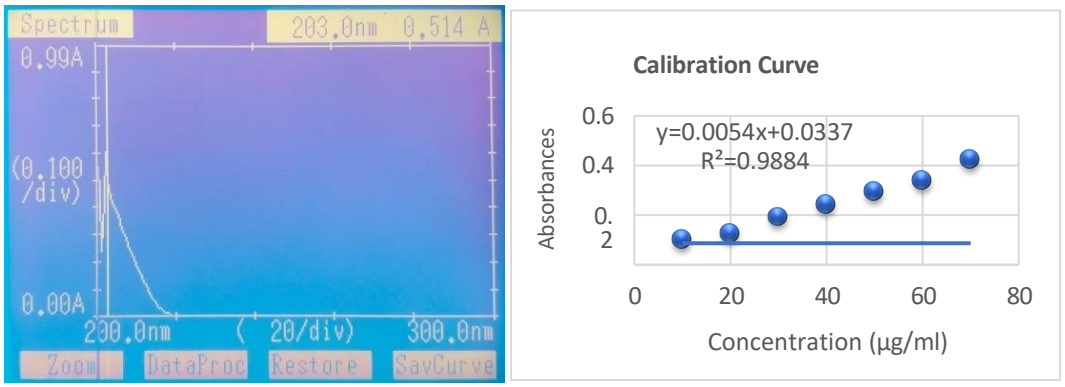

Figure1: UV graph and Calibration curve of Beta-Sitosterol (203.0nm)

i. Standard calibration curve

Table7: Calibration Curve of Beta-Sitosterolin Methanol

S. No	Concentration(µg/ml)	Mean Absorbance
1	10	0.103
2	20	0.128
3	30	0.194
4	40	0.245
5	50	0.297
6	60	0.341
7	70	0.426
Mean	0.247714286	
SD	0.116249322	
%RSD	46.96356275303644	

ii. Functional group identified by Infra-Red spectroscopy

Table 8: Interpretation of IRspectrum of Beta-Sitosterol

S. No.	Peak obtained	Reference peak	Functional group	Name of functional group
1	3372.26	3400-3300	N-H stretching	Aliphatic primary amine
2	2920.86	3000-2840	C-H stretching	Alkane
3	1648.60	1690-1640	C=N stretching	Imine/oxime
4	1340.29	1372-1335	S=O stretching	Sulfonate
5	1201.71	1205-1124	C-O	Tertiary

			stretching	alcohol
6	670.13	690-515	C-Br stretching	Halo compound
7	530.37	600-500	C-Istretching	Halo compound

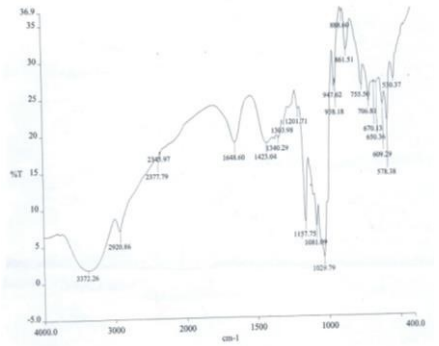

Figure 3: FTIR study of Beta-Sitosterol

c. Optimization of formulation by design of expert (DOE) software

This component mixture was utilized in the experiment, which produced 12 several batches of microsphere formulation. Numerous microsphere lots were created and there after valuated for every reaction, as mentioned. After fitting the observed responses to 12 runs, it was discovered that the linear model best fit the two dependent variables. Analysis of variance (ANOVA) is employed to a scerta in the model's significance when in contrast to other models. Every response was recorded for 12 runs, and the table shows the relationship between the variables that are independent and dependent.

i. BuildInformation

Table 9: Build in formation of DOE software

File Version	**12.0.1.0**		
StudyType	Response Surface	Subtype	Randomized
DesignType	Box-Behnken	Runs	12

ii. Formulation trial sasper Box–Behnken design

Table 10: Formulation trials

S. No	**FormulationCode**	**Polymer Eudragit S100 (mg)**	**Surfactant Tween 80 (%)**	**Sodium CMC (%)**	**Drug (mg)**	**Chloroform**	**Water (ml)**	**Stirring time**	**Particle size(nm)**	**Entrapment efficiency**
1	MS1	300	0.3	0.5	30	20	250	1	767.3	92.6
2	MS2	300	0.3	0.5	30	20	250	3	251.9	89.1
3	MS3	300	0.5	0.5	30	20	250	2	446.7	71.6
4	MS4	175	0.1	0.5	30	20	250	3	321.6	73.2
5	MS5	50	0.1	0.5	30	20	250	2	462.8	76.8
6	MS6	175	0.1	0.5	30	20	250	1	622.1	74.5
7	MS7	50	0.3	0.5	30	20	250	3	510.6	60
8	MS8	50	0.3	0.5	30	20	250	1	389.3	60.3
9	MS9	175	0.5	0.5	30	20	250	3	253.6	67.8

10	MS 10	50	0.5	0.5	30	20	250	2	253	62.3
11	MS 11	300	0.1	0.5	30	20	250	2	378.9	90.3
12	MS 12	175	0.5	0.5	30	20	250	1	400.2	68.5

3.3.4 FitSummary

Table11: Response1: Particle size

Source	Sequentialp-value	AdjustedR^2	PredictedR^2	
Linear	0.1789	0.2305	-0.2593	
2FI	0.0192	0.8046	0.4885	Suggested
Quadratic	0.0467	0.9577	0.8156	Aliased

d. Effect of formulation variables on Particle size (ANOVA for 2 FI model)

i. Response 1: Particle size

Table 12: Response 1: Particle size (ANOVA for 2 FI model)

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	2.449E+05	40810.62	8.55	0.0162	significant
A-Polymer	6560.85	6560.85	1.37	0.2939	
B-Surfactant	23317.20	23317.20	4.89	0.0781	
C-Stirringtime	88452.18	88452.18	18.53	0.0077	
AB	19265.44	19265.44	4.04	0.1008	
AC	1.013E+05	1.013E+05	21.23	0.0058	
BC	5921.30	5921.30	1.24	0.3160	
Residual	23865.96	4773.19			
CorTotal	2.687E+05				

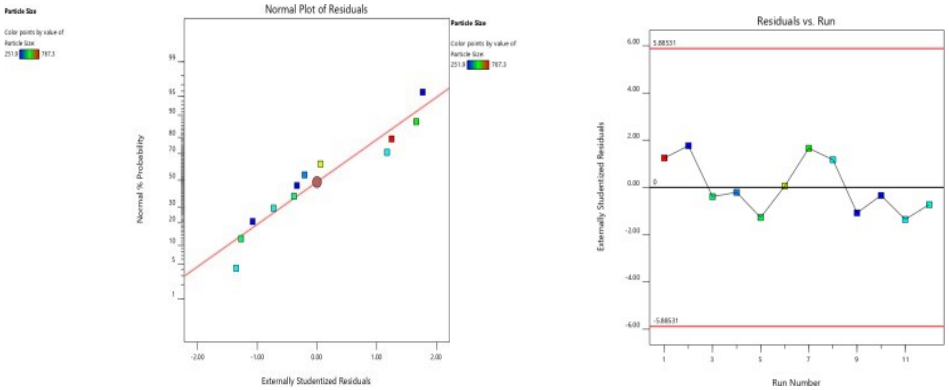

Figure 4: Graphical representation of Residuals vs run and Normal plot of Residuals and of microsphere formulation on particle size

ii. Predicted value and actual value of all formulations (Particle size and % EE)

Table 13: Predicted value and actual value of all formulations

Formulations	Actual Value of Particle size	Predicted Value of Particle size	Actual Value of % entrapment efficiency	Predicted Value of % entrapment efficiency
MS 1	767.30	714.46	92.60	85.17
MS 2	251.90	185.81	89.10	83.72

MS 3	446.70	465.55	71.60	78.87
MS 4	321.60	331.86	73.20	78.77
MS 5	462.80	516.25	76.80	68.97
MS 6	622.10	619.11	74.50	80.22
MS 7	510.60	446.89	60.00	62.67
MS 8	389.30	338.84	60.30	64.12
MS 9	253.60	300.84	67.80	67.62
MS 10	253.00	269.48	62.30	57.82
MS 11	378.90	434.73	90.30	90.02
MS 12	400.20	434.19	68.50	69.07

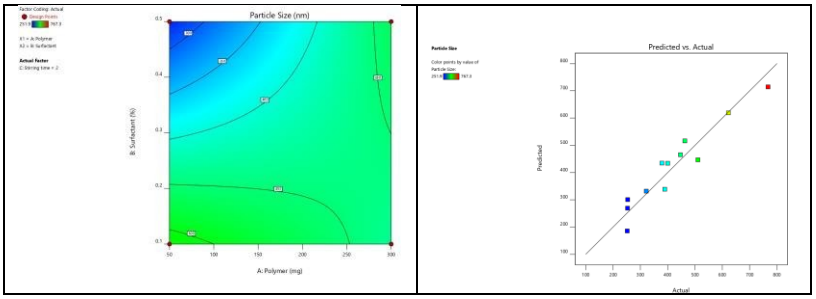

Figure 4: Two-dimensional contour plots for the effect of polymer and surfactant concentration on particle size.

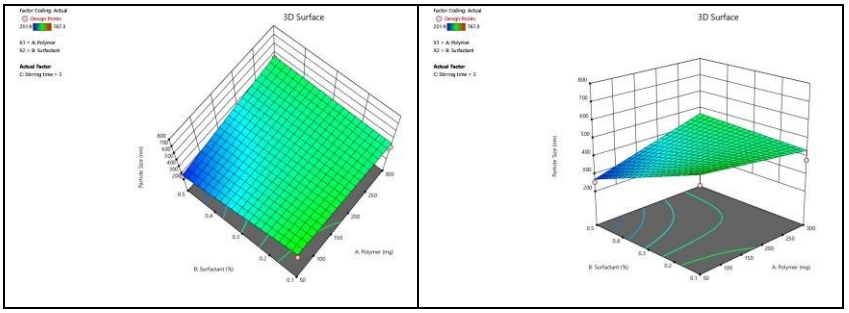

Figure 6: Response surface plot showing the combined effect of polymer and surfactant on particle size of microspheres.

ii. Effect of formulation variables on entrapment efficiency

Table 14: Response 2: Entrapment efficiency (Fit Summary)

Source	Sequential p-value	AdjustedR^2	PredictedR^2	
Linear	0.0044	0.7101	0.5257	Suggested
2FI	0.9882	0.5470	-0.1861	
Quadratic	0.7532	0.3749	-1.7276	Aliased

e. ANOVA for Linear model

i. Response 2: EE (ANOVA Linear model)

Table 15: Response 2: EE (ANOVA Linear model)

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	1139.05	379.68	9.98	0.0044	significant
A-Polymer	886.20	886.20	23.30	0.0013	
B-Surfactant	248.65	248.65	6.54	0.0338	
C-Stirring time	4.20	4.20	0.1106	0.7481	

Residual	304.28	38.04			
Cor Total	1443.34				

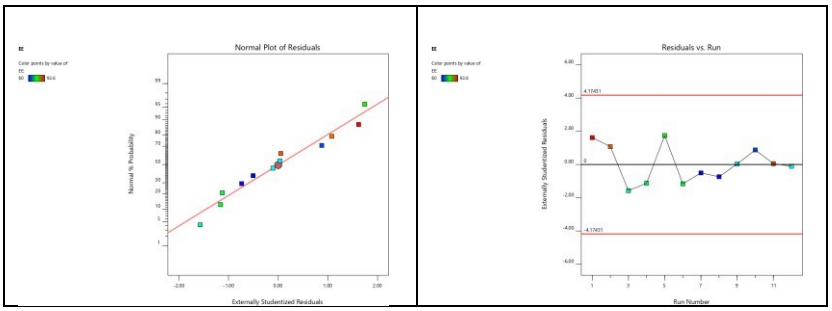

Figure 7: Graphical representation of residuals vs. run and normal plot of residuals of microsphere formulation on entrapment efficiency.

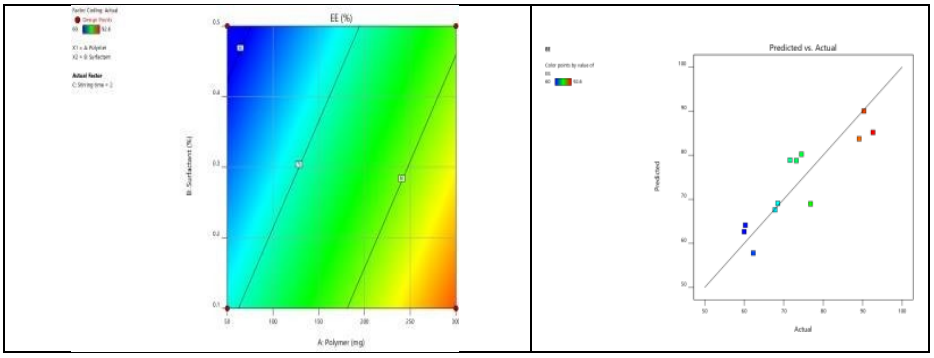

Figure 8: Two-dimensional contour plots for the effect of polymer and surfactant concentration on % entrapment efficiency.

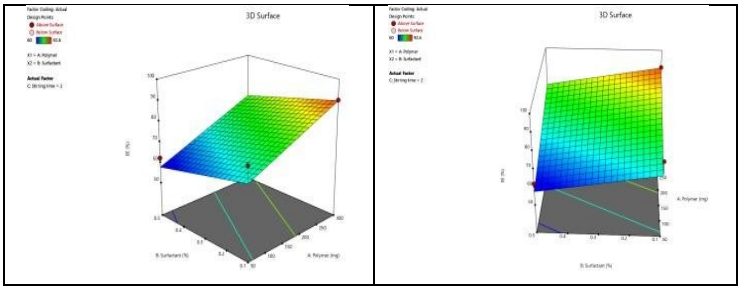

Figure 9: Response surface plot showing the combined effect of polymer and surfactant on entrapment efficiency of microsphere formulation (three-dimensional).

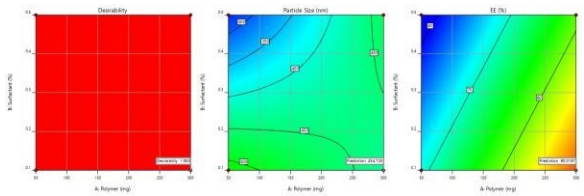

Figure 10: Response surface plot showing prediction data for optimization.

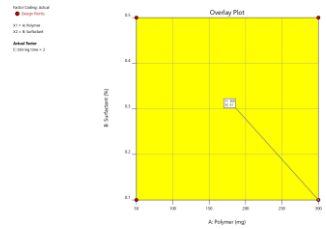

Figure 11: Overlay plot for optimization formulation.

ii. Optimized formula of microsphere formulation

Table 16: Optimized formula of microsphere formulation

S. No.	Polymer Eudrait	Surfactant Tween	Stirring time	Particle size	EE (%)	Desirability	

	S100 (mg)	80(%)		(nm)			
1	175.000	0.100	1.000	619.112	80.217	1.000	
2	**300.000**	**0.100**	**2.000**	**434.725**	**90.017**	**1.000**	**Selected**
3	50.000	0.300	3.000	446.887	62.667	1.000	

Table 17: Final composition of optimized microsphere formulation as per Design of Experiment approach.

S. No	Formulation Code	Polymer Eudragit S100 (mg)	Surfactant Tween 80 (%)	Sodium CMC (%)	Drug (mg)	Chloroform	Water (ml)	Stirring time
1	MS1	300	0.3	0.5	30	20	250	1

f. Characterization of optimized formulation

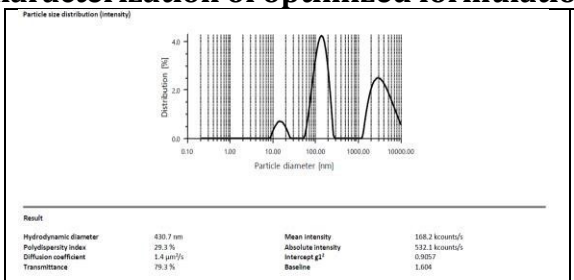

Figure12: Particle size

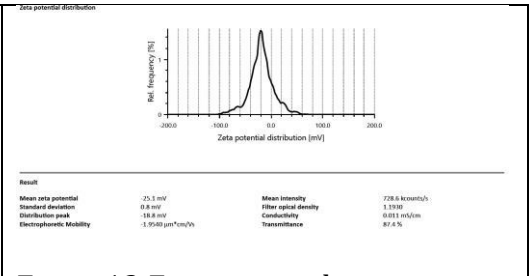

Figure13:Zetapotential

Table 18: Results of particle size, zeta potential, and entrapment efficiency.

S. No	Formulation	Particle size (Predicted value)	Particle size (Actual value)	Zeta potential	Entrapment efficacy (Predicted value)	Entrapment efficacy (Actual value)
1.	Microsphere	434.7	430.7nm	-25.1mV	90.01%	88.93%

Scanning Electron Microscope (SEM)

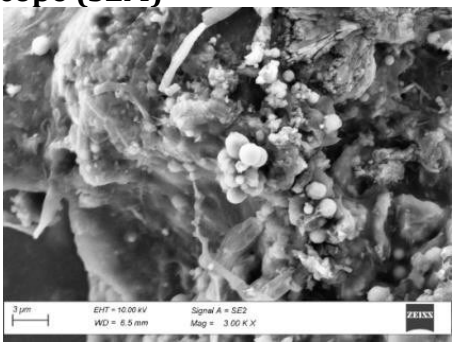

Figure 14: Scanning Electron Microscope (SEM) In-vitro drug release

Table 19: Release kinetics study of optimized formulation

Time (Hr)	cumulative % drug	% drug remainin	Square root	log Cumulative	log time	Log cumulative

	released	g	time	% drug remaining		% drug released
0	0	100	0.000	2.000	0.000	0.000
2	14.11	85.89	1.414	1.934	0.301	1.150
4	26.8	73.2	2.000	1.865	0.602	1.428
6	33.28	66.72	2.449	1.824	0.778	1.522
8	51.76	48.24	2.828	1.683	0.903	1.714
10	68.08	31.92	3.162	1.504	1.000	1.833
12	79.44	20.56	3.464	1.313	1.079	1.900
14	87.51	12.49	3.742	1.097	1.146	1.942
18	95.92	4.08	4.243	0.611	1.255	1.982

Correlation value

Table 20: Correlation value (R^2value)

Formulation	Model	Kinetic parameter values
Microsphere(optimized formulation)	ZeroOrder	R2=0.9706
	First Order	R^2=0.8905
	Higuchi	R^2=0.9263
	Korsmeyerpeppas	R^2=0.8908

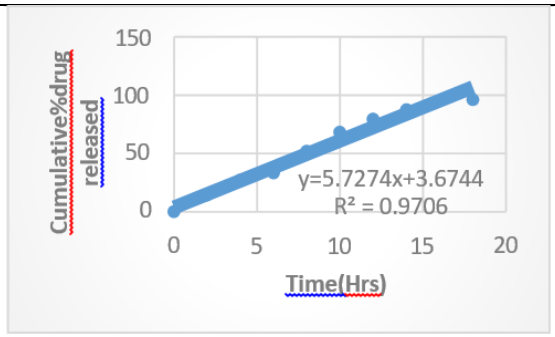

Figure 14: Zero Order Kinetic Model

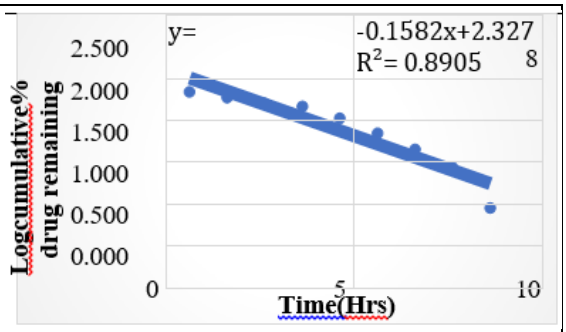

Figure 15: First Order kinetic model

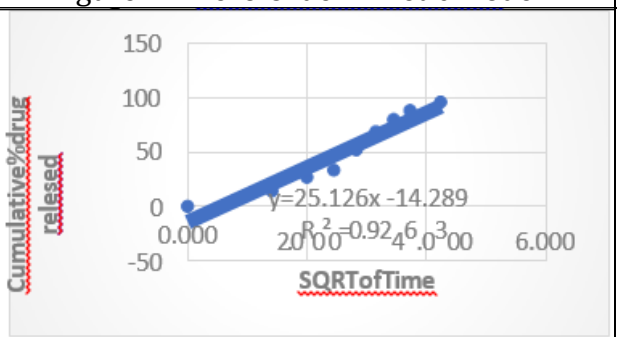

Figure 16: Higuchi model

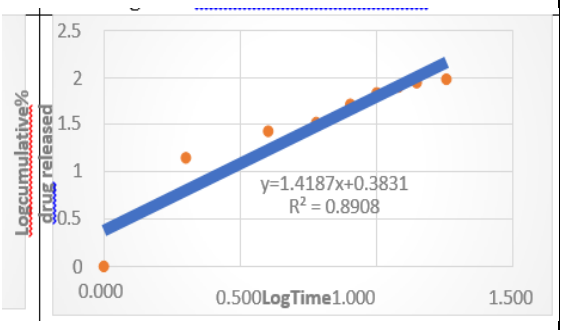

Figure 17: Korsmeyer peppas

Table 21: Antimicrobial Activity of Microsphere Formulation, Placebo Formulation, and Beta-Sitosterol Against E. coli

S. No.	SampleName	ZoneofInhibition(mm)
1.	OptimizedFormulation	16.0mm
2.	Placebo formulation	5.0mm
3.	Betasitosterol	13.0mm

f. Stability study

Table 22: Stability Study of Optimized Formulation (Microsphere)

S. No	Time (Days)	30⁰C±2⁰C and60±5% RH		40⁰C±2 ⁰C and70±5% RH	
		Particle Size(nm)	EE%	Particle Size(nm)	EE%
1.	0	430.7nm	88.93%	430.7nm	88.93%
2.	30	430.5nm	88.91%	430.1nm	88.93%
3.	45	430.5nm	88.90%	430.2nm	88.90%
3.	60	431.3nm	88.87%	430.5nm	88.86%
4.	90	431.1nm	88.89%	430.6nm	88.92%

4. Conclusion

The study concludes that Design of Expert (DOE) approach, which is aided by Quality by Design, is a successful method for optimizingthe manufacturingand formulation variables in the microsphere containing beta sitosterol. By determining and managing an ideal range of manufacturing and formulation factors, the QbD technique guarantees the ultimate product's quality. The predetermined product excellence is ensured by this methodical approach to the creation and design of medicinal formulations and manufacturing procedures. The study shows that thenumberof trials needed to generate acost-effective formula is decreased when QBD is applied in the optimization process. In pharmaceutical development, DOE integrates quality into both the process and the final product lowers the possibility of mistakes and flaws. This study's future potential includes evaluating the consequences of these approaches on the general quality and the products' security as well as applying DOE further in the creation of novel pharmaceutical formulations and manufacturing techniques.

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