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FORMULATION AND CHARACTERIZATION OF CHITOSAN MICROSPHERE AS A CARRIER OF SUNSCREEN AGENT

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ABSTRACT

Oxybenzone, a broad spectrum sunscreen agent widely used in the form of lotion and cream, has been reported to cause skin irritation, dermatitis, and systemic absorption. The objective of the present study was to formulation and characterization of chitosan microsphere as carrier of sunscreen agent. The formulation and characterization of an Oxybenzone-loaded microsphere drug delivery system were comprehensively studied and optimized using the Box–Behnken statistical design approach. The drug's solution pH was 7.8 (slightly alkaline), and the melting point was observed at 62°C, consistent with standard references, confirming its identity and purity. The Lambda max was found at 289.0 nm, UV spectrophotometric analyses. For particle size, the linear model was significant ($F = 14.33$, $p = 0.0014$), with a strong adjusted R^2 of 0.9842. For entrapment efficiency, the linear model also proved significant ($F = 14.92$, $p = 0.0012$), adjusted $R^2 = 0.7915$. In both responses had a statistically significant effect ($p < 0.0002$). The microsphere was spherical with Particle size in the range of 0.10–0.22 μm. The optimized formulation possesses the particle size of +519.96 + 61.22A – 35.36B – 299.41C and entrapment efficiency of +84.23 + 0.5112A – 0.2150B + 8.52C, respectively. This microsphere formulation of Oxybenzone may serve as a promising candidate for topical or cosmetic applications, offering controlled release and improved performance over conventional formulations.

KEYWORDS: Oxybenzone, chitosan microsphere, Entrapment efficiency, Lambda max.

1. INTRODUCTION

Sunscreens are topical formulations incorporating filters that protect our skin against ultraviolet radiation (UVR) emitted by the sun. Sunscreen use has been increasingly encouraged to protect against sunburn, skin cancer and photoaging that can occur because of prolonged and cumulative sun exposure **(Li *et al.*, 2019).** However, sunscreens and their constituent UVR filters have been purported to be problematic themselves. Sunscreen act by preventing and minimizing the damaging effects of the ultraviolet sun rays following exposure to the sunscreen have been demonstrated to increase the tolerance of the skin to uv exposure **(Saucedo *et al.*, 2020).**

Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microspheres matrix **(Lakshmi *et al.*, 2023).**

Chitosan is a polysaccharide comprising copolymers of glucosamine and N-acetyl glucosamine. Being biodegradable and biocompatible, chitosan has been used in the formulation of particulate drug delivery **(Qin and Zhao 2019).** Chitosan microspheres have been prepared by emulsion crosslinking, ion-induced coagulation, and spray-drying methods. Of these methods, the most common method used to prepare chitosan microspheres is the emulsion chemical crosslinking method. There are numerous reports on the use of glutaraldehyde (GA) as a crosslinking agent in the preparation of microspheres. The chemical crosslinking method for preparation of chitosan microspheres involves emulsification followed by crosslinking with a suitable crosslinking agent (e.g., GA) **(Patel and Patel 2014).**

Oxybenzone, a broad spectrum sunscreen agent widely used in the form of lotion and cream, has been reported to cause skin irritation, dermatitis, and systemic absorption. The present oxybenzone loaded sunscreen formulations have been reported to cause skin irritation,

photo allergic contact dermatitis, and systemic absorption **(DiNardo and Downs 2018).**

The present study was aimed towards the quality by design assisted formulation and characterization of chitosan microsphere as a carrier of sunscreen agent.

2. MATERIALS AND METHODS

2.1 Chemical

Oxybenzone was procured from Sihauli Chemicals Private Limited. Chitosan was received from Evonik Industries. Chloroform, DMSO and Petroleum ether was acquired from Rankem. Ethyl acetate was acquired from Shiva Chemical Co. Span 80 was received from Merck Millipore. All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Chemical Corporation Pvt Ltd**,** SRL AR Grade, Lab Chem Industries, Lab Reagent LR Grade and Astron Chemicals (India).

2.2 Pre-formulation study

These studies investigate critical parameters such as solubility, chemical stability, particle size, and compatibility with excipients, all of which influence the drug's therapeutic effectiveness, absorption, and shelf life **(Ainurofiq *et al.*, 2021).**

2.2.1 Organoleptic properties

This evaluation focused on examining the physical appearance, color, and odor of the drug substance to ensure its identity and suitability for further formulation **(Callemien and Collin 2009).**

2.2.2 Solubility study

Solubility is defined as the ability of a substance to dissolve uniformly at the molecular level within a solvent, forming a homogeneous system. In this study, the qualitative solubility of the drug was assessed in various solvents to determine its solubility profile. Accurately weighed drug samples (1 mg) were placed in 10 ml test tubes containing 1 ml of different solvents, including methanol, ethanol, chloroform, DMSO, water, and ethyl acetate. The extent of solubility (mg/ml) was evaluated through visual observation, providing preliminary data for selecting appropriate solvents during formulation **(Kerns *et al.*, 2008).**

2.2.3 Determination of pH

The pH was determined using a digital pH meter to evaluate its acidity or alkalinity, which is important for ensuring compatibility with formulation components **(Kumadoh *et al.*, 2024).**

2.2.4 Determination of Melting point

The melting point provides valuable information about the purity and crystalline nature of a drug sample **(Shrivastava and Shrivastava 2024).**

2.2.5 Determination of Maximum Wavelength (λ max)

- **Lambda max**

A stock solution was prepared by transferring 2 ml of the sample into a 10 ml volumetric flask, then diluting to the mark with methanol to achieve a concentration of 20µg/ml. This standard working solution was analyzed using UV spectroscopy in the wavelength range of 200 to 400 nm, with distilled water serving as the blank. The absorbance peaks were recorded, and the wavelength corresponding to the maximum absorption (λmax) was identified for further analysis **(Karabaliev *et al.*, 2021).**

- **Standard calibration curve**

An accurately weighed 100 mg of oxybenzone was transferred into a 100 mL volumetric flask and dissolved in 80% methanol. The volume was then adjusted to the mark with the same solvent. From this stock solution, 1 mL was pipetted into a 10 mL volumetric flask and diluted with methanol to prepare the working standard solution. This solution was scanned using a UV spectrophotometer over the wavelength range of 200–800 nm to identify the maximum absorption wavelength (λmax), which was then used for further quantitative analysis **(Sikder *et al.*, 2018).**

- **Linearity and Calibration Curve**

Starting with a 5 µg/mL working standard solution, a series of dilutions were prepared to yield concentrations of 5, 10, 15, 20, 25 and 30µg/mL. Aliquots of the stock solution were accurately transferred into 5 mL volumetric flasks, and the volume was made up to the mark with methanol. The absorbance of each diluted solution was recorded at 289.0 nm using distilled water as the blank. A calibration curve was constructed by plotting the absorbance values against their respective concentrations, producing a linear relationship for drug across the tested concentration range **(Van *et al.*, 2002).**

2.2.6 Functional group identified by FTIR

Fourier Transform Infrared (FTIR) spectroscopy is widely used in academic and pharmaceutical research to identify molecular structures and analyze the composition of complex mixtures. In this study, the FTIR spectra of oxybenzone-loaded microspheres were recorded using the KBr pellet technique over the range of 400–4000 cm^{-1}. The KBr pellets were prepared by mixing 1 mg of oxybenzone with 100 mg of spectroscopic grade KBr, which was dried using an IR lamp. This method enabled the identification of functional groups and the assessment of any chemical interactions between oxybenzone and the polymer matrix (Enders *et al.*, 2021).

2.3 Formulation of Microsphere

Preparation of microspheres Weigh amount chitosan and 300 mg of drug was dissolved in 15 ml 5% acetic acid. The drug-polymer dispersion was added in a 150 ml liquid paraffin (100 ml light liquid paraffin + 50 ml heavy liquid paraffin) containing 1.5 ml span 80 and it

was stirred with the help of mechanical stirrer at 1,500 revolutions per minute (rpm). After 10 and 40 min, GA (25% aqueous solution) was added and stirred continuously till 2 h. Suspension of chitosan microspheres in paraffin oil, thus obtained was allowed to stand for 15 min to let the microspheres settle down under gravity. Supernatant was decanted and filtered. The microspheres were dried at 40°C for 24 h. A total of nine batches, each in triplicate, were prepared as per the factorial design (32). The amount of crosslinking agent and drug: polymer ratio was varied in batch no CH1 to CH9 **(Patel and Patel 2014).**

Table 1: Composition of Microsphere Formulation.

Formulation code	Polymer Chitosan	Span 80 (ml)	(100 ml light liquid paraffin + 50 ml heavy liquid paraffin) (ml)	acetic acid (5%) ml	Drug (3%) mg	Glutaraldehyde (ml)	Stirring time (Hrs.)
CMSF 1	200	1.5	150.0	15.0	300	5	2
CMSF 2	500	1.5	150.0	15.0	300	3.5	1
CMSF 3	200	1.5	150.0	15.0	300	3.5	3
CMSF 4	500	1.5	150.0	15.0	300	5	2
CMSF 5	350	1.5	150.0	15.0	300	5	1
CMSF 6	350	1.5	150.0	15.0	300	2	1
CMSF 7	200	1.5	150.0	15.0	300	2	2
CMSF 8	500	1.5	150.0	15.0	300	3.5	3
CMSF 9	350	1.5	150.0	15.0	300	5	3
CMSF 10	500	1.5	150.0	15.0	300	2	2
CMSF 11	200	1.5	150.0	15.0	300	3.5	1
CMSF 12	350	1.5	150.0	15.0	300	2	3

2.4 Design of experiment

Using Design of Experiment (Version 12.0.1.0) software, the experiment was designed for the microsphere formulation. Design of experiments for optimization: Microspheres of oxybenzone were made and evaluated using "Box-Behnken experimental design". Aim of this study is to statistically maximize the parameters of the formulation of oxybenzone loaded microspheres to achieve optimal trapping and optimum particle size (**Abdallah, 2014).**

2.4.1 Independent and Dependent variables

Table 2: Independent and Dependent variables.

Independent variables	Dependent variables
(A) Polymer (mg)	(R1) Particle size (nm)
(B) Glutaraldehyde (ml)	(R2) Entrapment efficiency (%)
(C) Stirring time (hrs.)	

2.4.2 Values of variables

Table 3: Values of variables.

Factor	Name	Units	Type	Minimum	Maximum	CodedLow	Coded High	Mean	Std. Dev.
A	Polymer	mg	Numeric	200.00	500.00	-1 ↔ 200.00	+1 ↔ 500.00	350.00	127.92
B	Glutaraldehyde	ml	Numeric	2.00	5.00	-1 ↔ 2.00	+1 ↔ 5.00	3.50	1.28
C	Stirring time	Hrs	Numeric	1.0000	3.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	0.8528

2.5 Evaluation parameter of Drug loaded microsphere

2.5.1 Particle size and Zeta potential

In this study, the particle size of the prepared microspheres and Zeta potential analysis was performed to determine the surface charge of the microspheres and the electrophoretic mobility of particles under an applied electric field, was measured using a Malvern Zetasizer (Malvern Instruments) **(Honary and Zahir, 2013).**

2.5.2 Scanning Electron Microscopic (SEM)

A scanning electron microscope (SEM) was employed to examine the surface morphology of the drug-loaded microspheres **(Ayaz *et al.*, 2020).**

2.5.3 Entrapment efficiency

Entrapment efficiency of the drug-loaded microspheres was determined using an indirect method. The microsphere suspension was subjected to centrifugation using a REMI Ultra Centrifuge at 15,000 rpm for 30 minutes to separate the free (unentrapped) drug from the microspheres **(Dhakar *et al.*, 2010).**

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

2.6 *In-vitro* drug release Study of chitosan microsphere

The in vitro drug release profile of the microsphere formulation was investigated using the dialysis bag diffusion technique. The microsphere formulation was

carefully loaded into a dialysis bag and immersed in a beaker containing 100 mL of phosphate buffer (pH 7.4). The beaker was placed on a magnetic stirrer, with the temperature maintained at 37 ± 2 °C and the stirring speed fixed at 100 rpm throughout the experiment to simulate physiological conditions. At predetermined time intervals, 2 mL samples were withdrawn from the medium and replaced with an equal volume of fresh phosphate buffer to maintain sink conditions.

The withdrawn samples were then analyzed using a UV-visible spectrophotometer at 289.0 nm after suitable dilution to determine the concentration of the released drug. The cumulative percentage of drug released was calculated for each time point **(Lim *et al.*, 2000).**

2.7 Stability studies

Stability is a critical aspect of pharmaceutical product development, defined as the ability of a drug or formulation to maintain its physical, chemical, therapeutic, and toxicological properties over time under specified storage conditions. Stability testing provides essential data on how the quality of a drug substance or drug product changes over time when exposed to environmental factors such as temperature, humidity, and light. This data helps in determining suitable storage conditions, retest periods, and the product's shelf life **(Sengupta *et al.*, 2018).**

3. RESULT AND DISCUSION

3.1 Pre-formulation study of drug

3.1.1 Organoleptic properties

Table 4: Organoleptic properties of Oxybenzone.

Drug	Organoleptic properties	Observation
Oxybenzone	Color	Pale yellow
	Odor	Characteristic odor
	Appearance	Crystalline powder
	State	Solid

The organoleptic evaluation of Oxybenzone, as presented in Table 3, indicates that the drug exhibits a pale yellow color, a characteristic odor, and a crystalline powder appearance, confirming its typical physical properties.

3.1.2 Solubility study

Table 5: Solubility study of Oxybenzone.

Drug	Solvents	Observation/Inference
Oxybenzone	Water	Poorly soluble
	Ethanol	Soluble
	Methanol	Soluble
	Chloroform	Freely Soluble
	DMSO	Highly soluble

The results showed that oxybenzone is poorly soluble in water, which may limit its direct use in aqueous formulations without solubilizing agents. However, it demonstrated good solubility in ethanol and methanol, indicating its compatibility with organic solvents commonly used in pharmaceutical preparations. It was found to be freely soluble in chloroform and highly soluble in DMSO, suggesting these solvents could be effectively used in extraction, analytical procedures, or as carriers in non-aqueous systems.

3.1.3 pH, melting point and Lambda max determination

Table 6: pH melting point and Lambda max of Oxybenzone.

Drugs	Observed (pH)	UV absorption maxima (Lambda max)	Observed (Melting Point)	Reference (Melting Point)
Oxybenzone	7.8	289.0 nm	62°C	62°C-65°C

The pH of the Oxybenzone solution was measured to be 7.8, indicating that it is slightly alkaline. The observed melting point of Oxybenzone was 62°C, which falls within the reported reference range of 62°C to 65°C and Lambda max of oxybenzone was found to be 289.0 nm, which is consistent with standard values reported in the literature.

3.1.4 Calibration curve of Oxybenzone

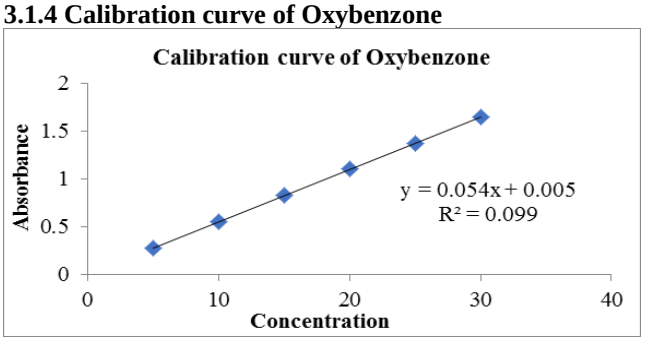

Figure 1: Calibration curve of Oxybenzone.

The calibration curve of Oxybenzone showed a linear increase in absorbance with rising concentrations ranging from 5 to 30 µg/mL, indicating good adherence to Beer's Law within this range. The mean absorbance was 0.960833 with a standard deviation of 0.510749. Despite this, the overall trend supports the use of this curve for quantitative analysis, though improvements in precision may be needed.

3.1.5 Functional group identified by Infra-Red spectroscopy

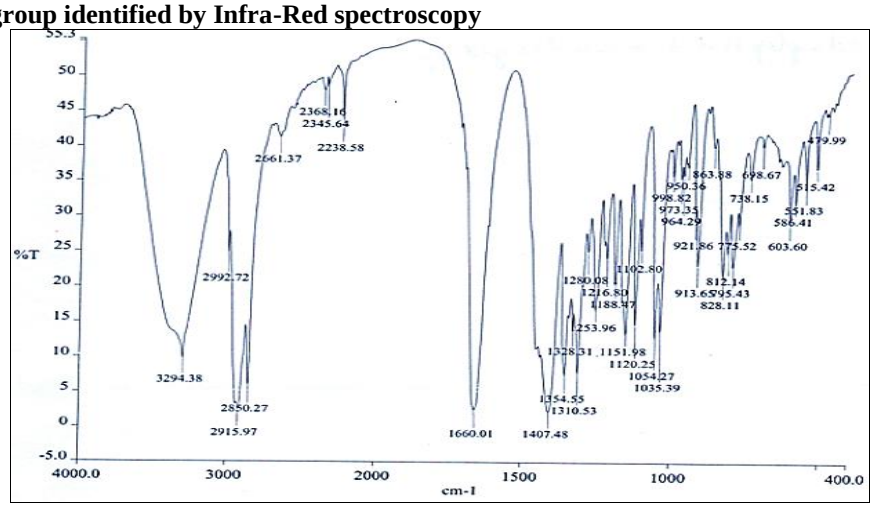

Figure 2: FTIR study of Oxybenzone.

Table 7: Interpretation of IR spectrum of Oxybenzone.

Peak obtained	Reference peak	Functional group	Name of functional group
3294.38	3330-3250	N-H stretching	Aliphatic primary amine
2238.58	2260-2222	CΞN Stretching	Nitrile
1660. 01	1662-1626	C=C Stretching	Alkene
1407.48	1410-1380	S=O stretching	Sulfonyl chloride
1280. 08	1310-1250	C-O stretching	Aromatic ester
1120.25	1124-1087	C-O stretching	Secondary alcohol
1035.39	1070-1030	S=O stretching	Sulfoxide

3.2 Optimization of formulation by design of expert (DOE) software

The formulation experiment involved a specific component mixture, resulting in the preparation of 12 distinct batches of microsphere. Each batch was evaluated for the designated responses. Upon analyzing the data from 12 experimental runs, it was determined that a linear model provided the best fit for both dependent variables. To evaluate the model's statistical significance compared to alternative models, an Analysis of Variance (ANOVA) was conducted. All responses were recorded across the 12 runs, and the tables illustrate the correlation between the independent and dependent variables.

3.2.1 Build Information

Table 8: Build information of DOE software.

File Version	12.0.1.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	12
Design Model	Quadratic	**Blocks**	No Blocks

3.2.2 Independent and Dependent variables

Table 9: Variables showing for optimization process.

Variables	Coding	Variables
Independent variables	A	(A) Polymer (mg)
	B	(B) Glutaraldehyde (ml)
	C	(C) Stirring time (Hrs)
Dependent variables	R1	Particle size (nm)
	R2	Entrapment efficiency (%)

3.2.3 Formulation trials as per Box–Behnken design

Table 10: Formulation trials.

Formulation code	Polymer Chitosan	Span 80 (ml)	(100 ml light liquid paraffin + 50 ml heavy liquid paraffin) (ml)	acetic acid (5%) ml	Drug (3%) mg	Glutaraldehyde (ml)	Stirring time	Particle size (nm)	Entrapment efficiency (%)
CMSF 1	200	1.5	150.0	15.0	300	5	2	265.31	83.21
CMSF 2	500	1.5	150.0	15.0	300	3.5	1	902.5	78.42
CMSF 3	200	1.5	150.0	15.0	300	3.5	3	192.46	94.76
CMSF 4	500	1.5	150.0	15.0	300	5	2	417.33	86.22
CMSF 5	350	1.5	150.0	15.0	300	5	1	778.23	72.11
CMSF 6	350	1.5	150.0	15.0	300	2	1	875.16	77.53
CMSF 7	200	1.5	150.0	15.0	300	2	2	328.38	79.23
CMSF 8	500	1.5	150.0	15.0	300	3.5	3	251.69	95.07
CMSF 9	350	1.5	150.0	15.0	300	5	3	376.2	89.47
CMSF 10	500	1.5	150.0	15.0	300	2	2	640.25	80.33
CMSF 11	200	1.5	150.0	15.0	300	3.5	1	935.88	78.75
CMSF 12	350	1.5	150.0	15.0	300	2	3	276.13	95.64

3.2.4 Limits of Variables (Constraints)

Table 11: Variables operating range for microsphere formulation.

Name	Goal	Lower Limit	Upper Limit	Importance
A: Polymer	is in range	200	500	3
B: Glutaraldehyde	is in range	2	5	3
C: Stirring time	is in range	1	3	3
Particle size	none	192.46	935.88	3
Entrapment efficiency	none	72.11	95.64	3

3.2.5 Fit Summary

Table 12: Response 1: Particle size.

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0014**	**0.9842**	**0.9469**	**Suggested**
2FI	0.8600	0.6994	0.2130	
Quadratic	0.2900	0.7805	0.0423	Aliased

3.3 Effect of formulation variables on Particle size (ANOVA for Linear model)

3.3.1 Response 1: Particle size

Table 13: Response 1: Particle size (ANOVA for Linear model).

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	7.572E+05	2.524E+05	14.33	0.0014	significant
A-Polymer	29980.66	29980.66	1.70	0.2283	
B-Glutaraldehyde	10000.52	10000.52	0.5677	0.4728	
C-Stirring time	7.172E+05	7.172E+05	40.71	0.0002	
Residual	1.409E+05	17616.99			
Cor Total	8.981E+05				

Factor coding is **coded.** Sum of squares is **Type III – Partial.** The Model F-value of 14.33 implies the model is significant. There is only a 0.14% chance that an F-value this large could occur due to noise.

Table 14: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	519.96	38.32	431.60	608.32	
A-Polymer	61.22	46.93	-47.00	169.43	1.0000
B-Glutaraldehyde	-35.36	46.93	-143.57	72.86	1.0000
C-Stirring time	-299.41	46.93	-407.62	-191.20	1.0000

Final Equation in Terms of Coded Factors
Particle Size= +519.96 Intercept +61.22X1 Polymer (A)- 35.36 Surfactant X2 (B) -299.41stirring time X3 (C)

Coded equation= +519.96 +61.22 AX1- 35.36 BX2 -299.41 CX3.

3.3.2 Predicted value and actual value of Particle Size and % EE

Table 15: 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
CMSF 1	265.31	423.39	83.21	83.50
CMSF 2	902.50	880.59	78.42	76.22
CMSF 3	192.46	159.33	94.76	92.23
CMSF 4	417.33	545.82	86.22	84.52
CMSF 5	778.23	784.02	72.11	75.50
CMSF 6	875.16	854.73	77.53	75.93
CMSF 7	328.38	494.10	79.23	83.93
CMSF 8	251.69	281.77	95.07	93.26
CMSF 9	376.20	185.19	89.47	92.53
CMSF 10	640.25	616.53	80.33	84.95
CMSF 11	935.88	758.15	78.75	75.20
CMSF 12	276.13	255.91	95.64	92.96

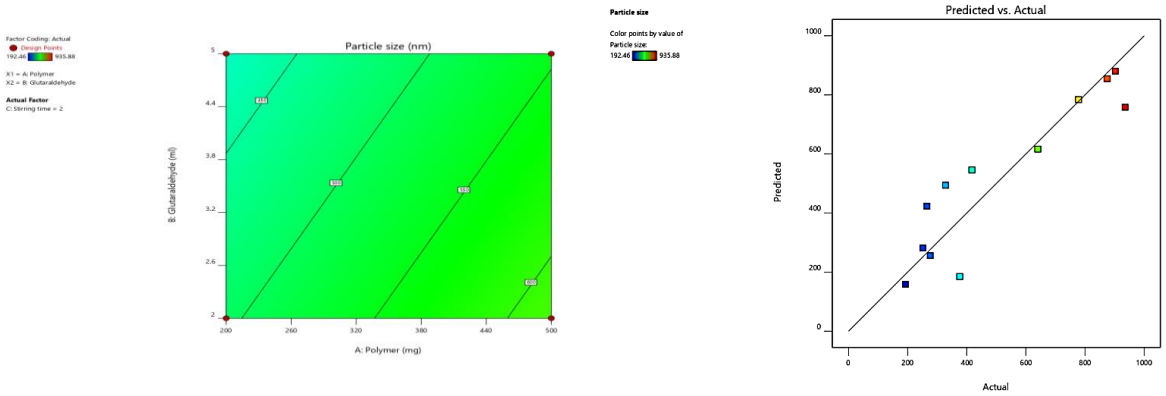

Figure 3:Two-dimensional contour plots for the effect of polymer and surfactant concentration on particle size.

3.3.3 Effect of formulation variables on Entrapment efficiency

Table 16: Response 2: Entrapment efficiency (Fit Summary).

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**0.0012**	**0.9915**	**0.9588**	**Suggested**
2FI	0.9961	0.6701	0.1363	
Quadratic	0.4523	0.6760	-0.4137	Aliased

3.4 ANOVA for Linear model

3.4.1 Response 2: EE (ANOVA Linear model)

Table 17: Response 2: EE (ANOVA Linear model).

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	582.67	194.22	14.92	0.0012	significant
A-Polymer	2.09	2.09	0.1606	0.6991	
B-Glutaraldehyde	0.3698	0.3698	0.0284	0.8703	
C-Stirring time	580.21	580.21	44.57	0.0002	
Residual	104.14	13.02			
Cor Total	686.81				

Factor coding is **coded.** Sum of squares is **Type III – Partial.** The **Model F-value** of 14.92 implies the model is significant. There is only a 0.12% chance that an F-value this large could occur due to noise. **P-values** less than 0.0500 indicate model terms are significant. In this case C is a significant model term.

Table 18: Coefficients in Terms of Coded Factors.

Factor	Coefficient Estimate	Standard Error	95% CI Low	95% CI High	VIF
Intercept	84.23	1.04	81.83	86.63	
A-Polymer	0.5112	1.28	-2.43	3.45	1.0000
B-Glutaraldehyde	-0.2150	1.28	-3.16	2.73	1.0000
C-Stirring time	8.52	1.28	5.57	11.46	1.0000

Final Equation in Terms of Coded Factors

Entrapment efficiency= +84.23 Intercept + 0.5112X1A-0.2150 X2B +8.52X3C. For certain amounts of each element, predictions regarding the reaction can be made using the equation expressed in terms of coded factors. By default, the components' high and low levels are denoted by the numbers +1 and -1, respectively.

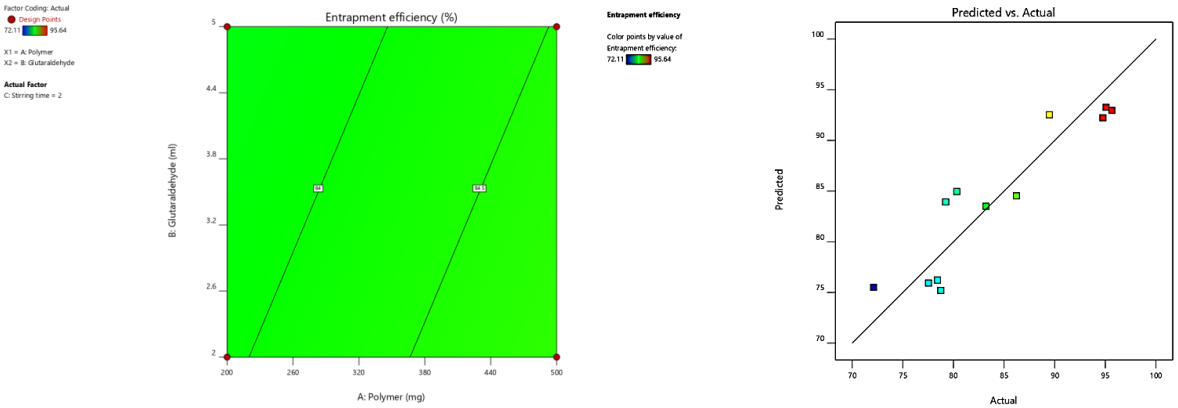

Figure 4: Two-dimensional contour plots for the effect of polymer and surfactant concentration on % entrapment efficiency.

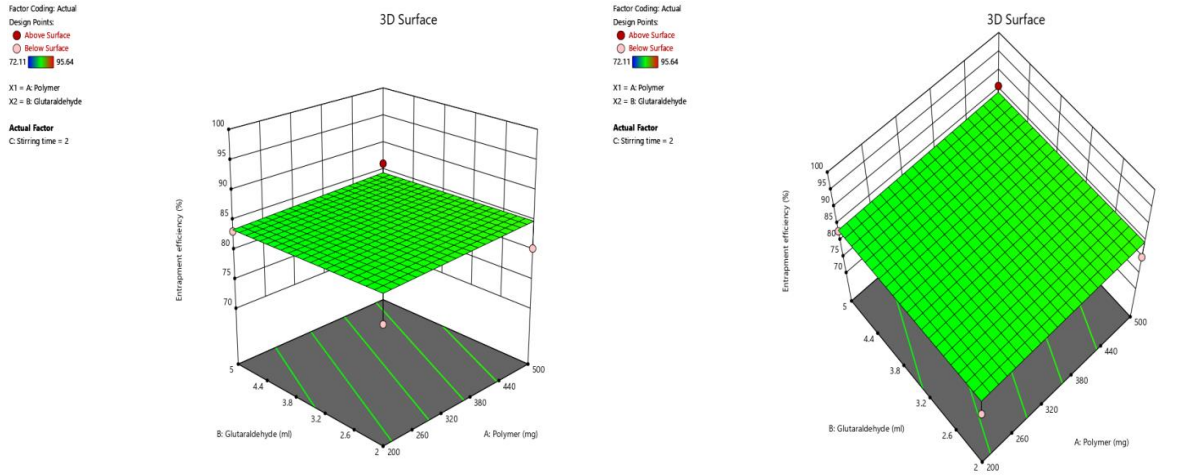

Figure 5: Response surface plot showing combined effect of Polymer and surfactant on entrapment efficiency of microsphere formulation (Three dimensional).

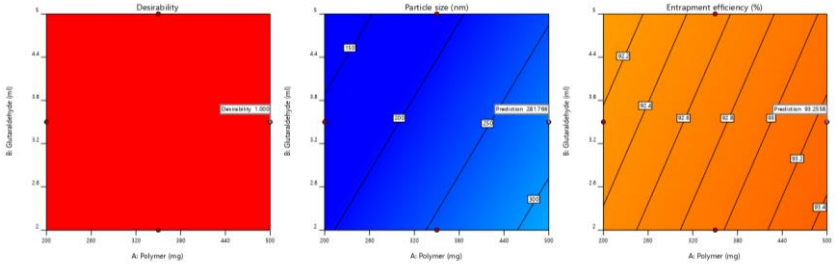

Figure 6: Response surface plot showing prediction data for optimization.

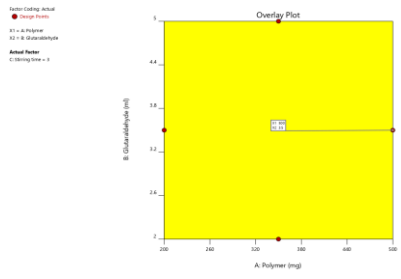

Figure 7: Overlay plot for optimization formulation.

3.4.2 Optimized formula of microsphere formulation

Table 19: Optimized formula of microsphere formulation.

S.No	Polymer	Glutaraldehyde	Stirring time	Particle size	Entrapment efficiency	Desirability	
1.	**500.000**	**3.500**	**3.000**	**281.766**	**93.256**	**1.000**	**Selected**
2.	200.000	3.500	3.000	159.331	92.233	1.000	
3.	350.000	5.000	1.000	784.015	75.497	1.000	

Table 20: Final Composition of optimized Microsphere formulation as per Design of experiment approach.

Formulation code	Polymer Chitosan (mg)	Span 80 (ml)	(100 ml light liquid paraffin + 50 ml heavy liquid paraffin) (ml)	acetic acid (5%) ml	Drug (3%) mg	Glutaraldehyde (ml)	Stirring time (hrs.)
OMSF	500.000	0.3	0.5	30	20	3.500	3.000

3.5 Characterization of optimized formulation

Table 21: Particle size and zeta potential.

Formulation	Particle size (Predicted value)	Particle size (Actual value)	Zeta potential
Microsphere	281.76 nm	274.90 nm	- 27.8 mV

3.5.1 Particle Size and Zeta potential analysis

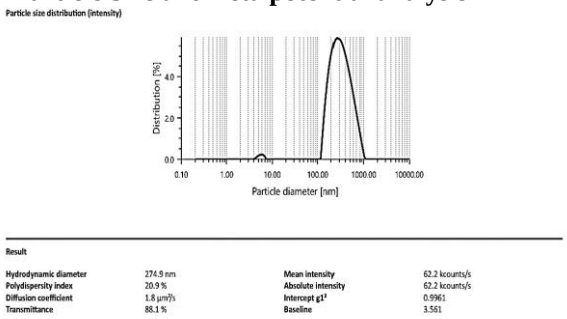

Figure 8: Particle Size.

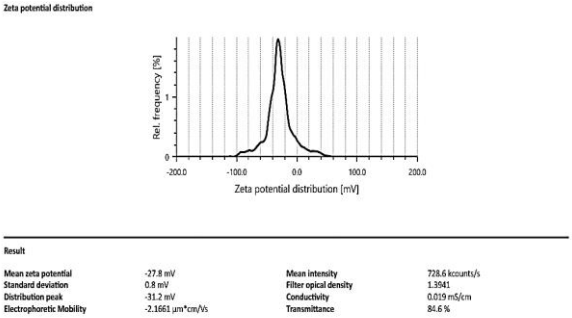

Figure 9: Zeta Potential.

3.5.2 Entrapment efficacy

Table 22: Entrapment efficacy.

Formulations	Entrapment efficacy (Predicted value)	Entrapment efficacy (Actual value)
Microsphere	93.25 %	91.86%

3.5.3 SEM Analysis

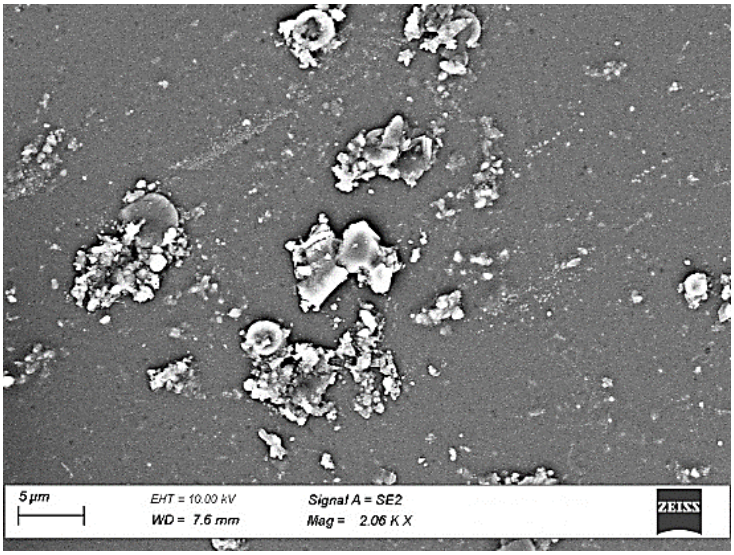

Figure 10: SEM of optimized Microsphere formulation.

3.6 *In-vitro* drug release

Table 23: Release kinetics study of optimized formulation.

Time (Hr)	cumulative % s drug released	% drug remaining	Square root time	log Cumu % drug remaining	log time	Log cumulative %drug released	% Drug released
0	0	100	0.000	2.000	0.000	0.000	100
1	23.11	76.89	1.000	1.886	0.000	1.364	23.11
2	36.1	63.9	1.414	1.806	0.301	1.558	12.99
4	43.13	56.87	2.000	1.755	0.602	1.635	7.03
6	57.59	42.41	2.449	1.627	0.778	1.760	14.46
8	66.83	33.17	2.828	1.521	0.903	1.825	9.24
10	77.17	22.83	3.162	1.359	1.000	1.887	10.34
12	82.41	17.59	3.464	1.245	1.079	1.916	5.24
13	97.14	2.86	3.606	0.456	1.114	1.987	14.73

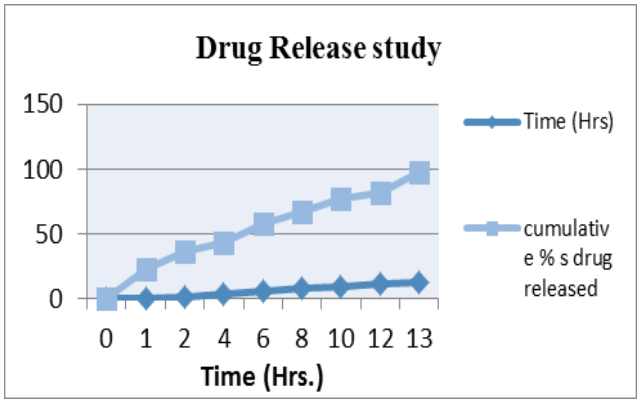

Figure 11: Release kinetics study of optimized formulation.

3.6.1 Correlation value

Table 24: Correlation value (R^2 value).

Formulation	Model	Kinetic parameter values
Microsphere (optimized formulation)	Zero Order	R2 = 0.9795
	First Order	R^2 = 0.8126
	Higuchi	R^2 = 0.9688
	Korsmeyerpeppas	R^2 = 0.8104

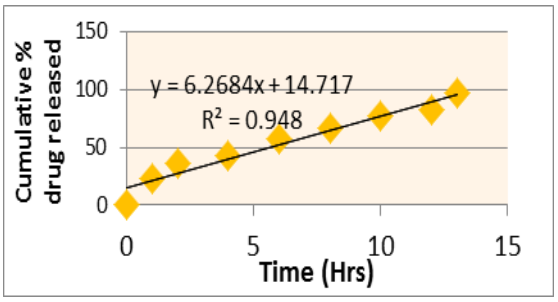

Figure 12: Zero order kinetic model.

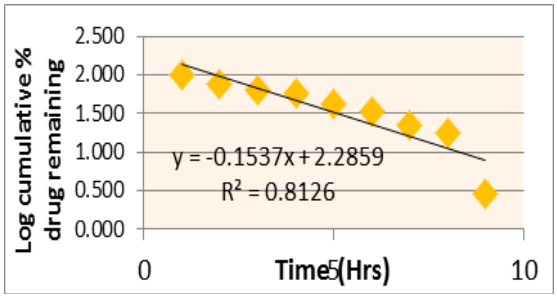

Figure 13: First Order kinetic model.

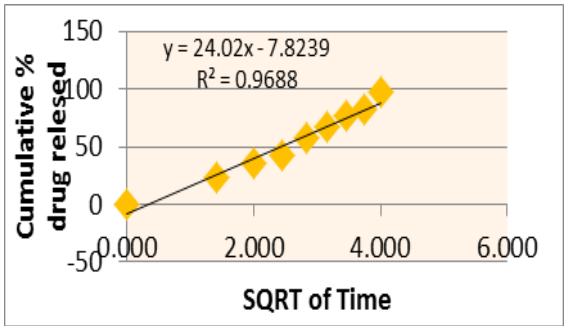

Figure 14: Higuchi model.

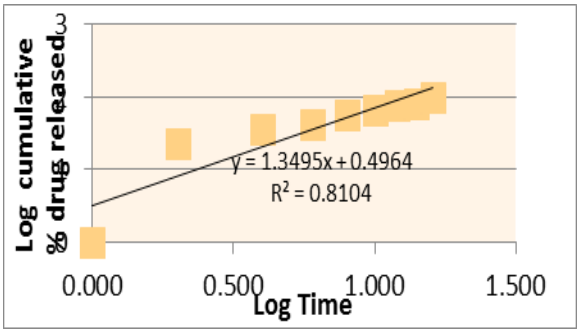

Figure 15: Korsmeyer peppas.

3.7 Stability study

Table 25: Stability Study of optimized formulation (Microsphere).

Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH		40^0C±2 ^{0}C and 70 ±5% RH	
	Particle Size (nm)	EE %	Particle Size (nm)	EE %
0	274.90	91.86%	274.90 nm	91.86%
30	274.88	91.85	274.85	91.79
60	274.93	91.82	274.80	91.80
90	274. 96	91.82	274.81	91.82

4. CONCLUSION

A stable and effective Oxybenzone microsphere formulation was successfully developed and optimized using a Box–Behnken design. Key findings include: Optimized particle size (275 nm) supports efficient skin penetration and sustained release. High entrapment efficiency (92%) ensures effective drug loading. Zero-order release kinetics suggests prolonged drug release potential. Good zeta potential (-27.8 mV) confirms physical stability. Stability studies validate long-term storage potential under accelerated conditions.

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