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"Quality by Design assisted Formulation, Development and Optimization of Nano-sponges by Design of Experiment (DOE) Approach"

Ojasvi Dubey*[1], Amit sahu[2], S. Nayak[3]

[1]Student, Bansal College of pharmacy, Bhopal (Madhya Pradesh)

[2]Associate professor, Bansal College of pharmacy, Bhopal (Madhya Pradesh)

[3]Principal, Bansal College of pharmacy, Bhopal (Madhya Pradesh)

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KEYWORDS

Nanosponge, Quality by Design (QbD), Berberine, zeta potential, Entrapment efficiency.

ABSTRACT:

This study describes the integration of Quality by Design (QbD) principles in the formulation and development of topical delivery systems represents a significant advancement in pharmaceutical sciences, particularly for enhancing the efficacy and safety of active ingredients. This study applies Quality by Design (QbD) principles to develop a nanosponges gel system incorporating Berberine. Nanosponges enhance drug stability, control release, and improve skin permeation. Using Design of Experiments (DoE), key formulation parameters such as polymer concentration, surfactant type, and drug load were optimized. The gel was tested for particle size, zeta potential, drug entrapment, and release profiles, with stability and efficacy assessed in real-world conditions. A double beam UV visible spectrophotometer (Shimadzu-1700) was utilized to ascertain a substance's lambda max, or absorption maxima. The lambda max of the Berberine was found to be 355.0 nm. The Berberine pH of 6.6 was discovered to be well within the parameters of the medication's specification. The optimized formulations yielded an average particle size of 181.04 nm. The optimized nanosponges formulation's zeta potential values (-29.0 mV) showed that the nanosponges were stable. The prepared optimized nanosponges possess high drug entrapment efficiency and found to be in the range of 90.18%. The QbD approach successfully produced a stable, efficient formulation, demonstrating its value in advancing topical drug development.

1. INTRODUCTION

DOE is a statistical methodology used to systematically plan, conduct, analyze, and interpret experiments to obtain valid and reliable results. It allows researchers to efficiently explore and identify the significant factors influencing a process or product's performance. DOE is an important statistical method used in controlling input factors or variables in order to ascertain the level of relationships with the output (responses), so as to ensure product or process quality.

Nanosponge is a new type of material with a cavity of a few nanometers in size, in which various substances can be encapsulated. These particles can carry lipophilic and hydrophilic substances and increase the solubility of poorly water-soluble molecules. poorly water-soluble molecules **(Panda *et al.,* 2015).** Nanosponge is a virus sized, naturally degradable scaffold like structure. The long polymer strands are mixed in solution with small molecules called cross-linking, which have affinity for certain parts of polymer **(Kapileshwari *et al.,* 2020).**

Nanosponges loaded with berberine are promising drug delivery systems that can enhance the therapeutic effects of this natural compound. Berberine, an alkaloid found in various plants, is known for its diverse pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant effects. Studies have shown that berberine chloride can inhibit cancer cell proliferation, induce apoptosis, and sensitize cancer cells to chemotherapy **(Majidzadeh *et al.,* 2020).** Its

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ability to target multiple signalling pathways involved in cancer development makes it a promising candidate for cancer treatment. The encapsulation of berberine chloride within gelatin/perfluorohexane nanodroplets improves its solubility and stability, allowing for better drug dispersion and preventing precipitation **(Givarian *et al.,* 2024).**

This study shows the Quality by Design (QbD) principles to assist the formulation and optimization of nanosponge containing barberine that advancing the tropical drug development.

2. MATERIALS AND METHODS

2.1 Chemical

Berberine was procured from Sigma-Aldrich Drugs Pvt Ltd. Beta cyclodextrin was received as sample from Himedia laboratories pvt. ltd, Mumbai (India). Di-phenyl carbonate was acquired from Spectrochem Pvt Ltd (India). All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Fine Chem Ltd., Mumbai

2.2 Pre-formulation study of Berberine

For Berberine compounds, the following pre-formulation investigations were carried out **(Ahirwar and Shukla 2023).**

2.2.1 Organoleptic evaluation: 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 **(Ainurofiq *et al.,* 2021).**

2.2.2 Solubility study: Solubility testing was done to choose suitable solvent system to dissolve the drug. The medication (1 mg) was precisely weighed and put into a 10 ml test tube; then, it was dissolved in the respective solvents (1 ml each) such as Methanol, Ethanol, Chloroform, DMSO**,** water and PBS 7.4. The solubility (mg/ml) was observed by visual inspection **(Unde and Kurup 2021).**

2.2.3 Determination of pH : Digital pH meter was used to ascertain the pH of Berberine.

2.2.4 Determination of Melting point : The point of melting was determined by use of open Capillary method using capillary tube by filling the drug samples on one closed side of the capillary tube **(Shrivastava and Shrivastava 2024).**

2.2.5 Determination of Maximum Wavelength (λ max)

1. **Preparation of Berberine standard stock solution in methanol**

Standard solution of Berberine was prepared by dissolving accurately weighed 10 mg of Berberine in a 10 ml volumetric flask with 5 ml of PBS 7.4 solvent. The volume was made up to 10 ml with solvent 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 (Phosphate buffer 7.4) to acquire a solution that has a concentration 100 µg/ml which is standard stock solution **(Afsar *et al.,* 2016).**

2. **Lambda max**

A stock solution A 10 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 **(Desta and Amare 2017).**

3. **Linearity and Calibration Curve**

From the working standard solution of 100 µg/mL, range of dilutions that were made was 5, 10, 15, 20, 25, and 30 µg/ml. After precisely transferring the Berberine working standard stock solution into a series of 5 mL calibrated flasks, the volume was adjusted with solvent. At Berberine 355.00 nm, the absorbance of the resultant solutions was measured in comparison to a blank of solvent. A calibration curve was created by graphing the drug's absorbance against concentration. A six-point the calibration curve was produced for Berberine concentrations ranging from 5 to 30 µg/ml **(Sayed *et al.,* 2014).**

4. **Functional group identified by FTIR**

The Nanosponges' FTIR spectra were recorded by KBr press pellet technique and scanning from 400 – 4000cm-1. The KBr disc was made with 1 mg of Berberine in 100 mg of spectroscopic grade KBr which has been dried using IR lamp. The medication and KBr were combined, and the disc were formed by applying

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hydraulic pressure. This disc was placed in FT-IR chamber **(Sana, 2024).**

2.3 Formulation of β-cyclodextrin Nano sponges

Beta-cyclodextrin Nano sponges were prepared by 'hot melt method'. For one to six hours, the finely homogenized anhydrous polymer (β-CD) and cross linker (DPC) were heated gradually to 90 to 100ºC while being stirred by a magnetic device. The substrate mixture (β-CD and DMC) was allowed to react for 1 to 6 h so as to ensure completion of cross linking reaction amongst them; creating Nano sponges as a result. The one that obtained reaction mixture was subsequently cooled at room temperature. The solid thus obtained was washed repeatedly using double distilled water (to remove unreacted β-CD). Finally, the placebo Nano sponges obtained were dried (at 40 ºC) and stored in desiccator, till further use **(Kumar *et al.*, 2021).**

2.4 Loading of extract in Nano sponges

Drug was loaded in prepared Nano sponges by using a freeze-drying technique. Placebo NS (1 gm) was distributed with a magnetic stirrer in 50 millilitres of double-distilled water. Berberine (Drug) (30 mg) was added to above dispersion. The obtained dispersion was then, sonicated (for 10 minutes), and subsequently kept for 1 to 3 hours (under stirring). To separate the un-entrapped drug, which was a residue underneath the colloidal supernatant, the resultant suspensions were centrifuged for ten minutes. The supernatant was then lyophilized at -81°C under a pressure of 0.0010 mbar **(Ravi *et al.*, 2014).**

2.4.1 Composition of Nano sponges formulation

Table 1: Composition of Nano sponges formulation

Formulation code	Beta-cyclodextrin-Polymer (mg) X1	Diphenyl carbonate-Cross linker (DPC) (mg) X2	Distilled water (ml)	Stirring time (hrs) X3	Berberine (mg)	Temperature (ºC)
NSF 1	125	100	50	6	50	90 to 100
NSF 2	125	300	50	6	50	90 to 100
NSF 3	200	100	50	3.5	50	90 to 100
NSF 4	200	200	50	6	50	90 to 100
NSF 5	200	200	50	1	50	90 to 100
NSF 6	50	200	50	1	50	90 to 100
NSF 7	50	200	50	6	50	90 to 100
NSF 8	125	300	50	1	50	90 to 100
NSF 9	125	100	50	1	50	90 to 100
NSF 10	200	300	50	3.5	50	90 to 100
NSF 11	50	300	50	3.5	50	90 to 100
NSF 12	125	200	50	3.5	50	90 to 100
NSF 13	50	100	50	3.5	50	90 to 100

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Figure 1: Trial Batches of Nano sponges formulation

2.5 Design of experiment

Design of the experiment to formulate Nano sponges was performed by Design Expert (Version 12.0.1.0) software.

2.5.1 Independent and Dependent variables

Table 2: Independent and Dependent variables

Independent variables	Dependent variables
(X1) Polymer (Beta-cyclodextrin) (mg)	(Y1) Particle size (nm)
(X2) Cross linker (DPC) (mg)	(Y2) Zeta potential (mV)
(X3) Stirring time (hrs)	

2.5.2 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	200.00	-1 ↔ 50.00	+1 ↔ 200.00	125.00	61.24
B	Crosslinker	mg	Numeric	100.00	300.00	-1 ↔ 100.00	+1 ↔ 300.00	200.00	81.65
C	Stirring time	hrs	Numeric	1.0000	6.00	-1 ↔ 1.00	+1 ↔ 6.00	3.50	2.04

2.6 Characterization of Nano sponges formulation

2.6.1 Particle size: One of the most crucial parameters for describing a Nano sponge is its particle size. A Malvern Zeta sizer (Malvern Instruments) was used to measure the Nano sponge's size **(El-Assal *et al.*, 2019).** Using a Malvern zeta sizer, zeta potential calculates the surface charge of prepared Nano sponges **(Dhakar *et al.*, 2019).**

2.6.3 Scanning Electron Microscopic (SEM): SEM images of the optimized Nano sponges formulation were taken by scanning electron microscope **(Ahmed *et al.*, 2021).**

2.6.4 Drug entrapment efficiency: Determination of % entrapment efficiency weighs of drug loaded formulations were crushed, dissolved in 20 ml methanol, stirred for 60 minutes, the supernatant was withdrawn, diluted and analyzed spectrophotometrically at a wavelength of 355.0 nm (Jenway, UK) **(Huang, 2018).** The entrapment efficiency was calculated from the following equation:

% EE = The actual amount of drug/The theoretical amount of the drug * 1000

2.7 *In-vitro* drug release

The dialysis bag diffusion method was employed to look into the drug release in vitro of Nanosponges-loaded compositions. A dialysis bag was filled with the Nano sponges formulation and then put in a beaker containing 100 milliliters 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

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spectrophotometer was used to analyze the samples at 355.0 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 **(Liaqat *et al.*, 2025).**

2.8 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. 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. The drug loaded nanosponges formulation was packed and were positioned in the stability test chamber and exposed to stability studies at accelerated testing ($25^0C\pm2^0C$ and 60 ± 5% RH) and ($40^0C\pm2^0C$ and 70 ±5% RH) for 3 months. At intervals of 30, 45, 60, and 90 days (3 months), the formulation was examined for assessment parameters such as particle size and drug entrapment efficiency tests **(Sharma *et al.*, 2024).**

3. RESULT AND DISCUSION

3.1 Pre-formulation study of Berberine

Pre-formulation study aims to generate information that is beneficial for the formulation development personally by developing stable and bioavailable dosage forms. The following pre-formulation studies were performed for Berberine substances**.**

3.1.1 Organoleptic evaluation

The drug sample's physical characteristic, the parameter given below in table 4.

Table 4: Organoleptic evaluation of Berberine

Physical parameter	Observation
Color	Yellow color
Odor	Characteristic
Appearance	Solid powder

3.1.2 Solubility study

Table 5: Solubility study of Berberine

Drug	Solvents	Observation/Inference
Berberine	Water	Soluble
	Ethanol	Slightly soluble
	Methanol	Sparingly soluble
	Acetone	Soluble
	DMSO	Freely Soluble
	PBS 7.4	Freely soluble

From the results, it was observed that the drug is freely soluble in DMSO and PBS 7.4, and soluble in water and acetone.

3.1.3 Determination of pH and melting point

Table 6: pH and melting point of Berberine

Drugs	Observed (pH)	Observed (Melting Point)	Reference (Melting Point)
Berberine	6.6	202°C	204-206°C

The pH of the Berberine was found to be 6.6 which are well within the limits of the drug specification and the temperature at which the Berberine was melt discovered to be 202^0C.

3.2 Determination of λ max by UV spectroscopy

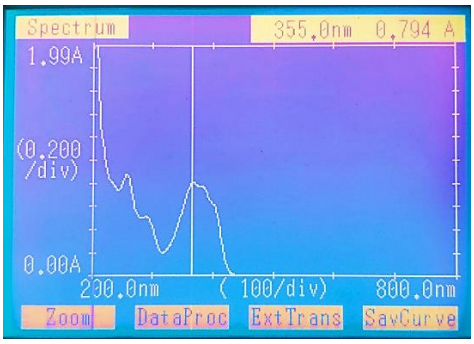

Figure 2: UV graph of Berberine (355.0 nm)

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3.2.1 Standard calibration curve

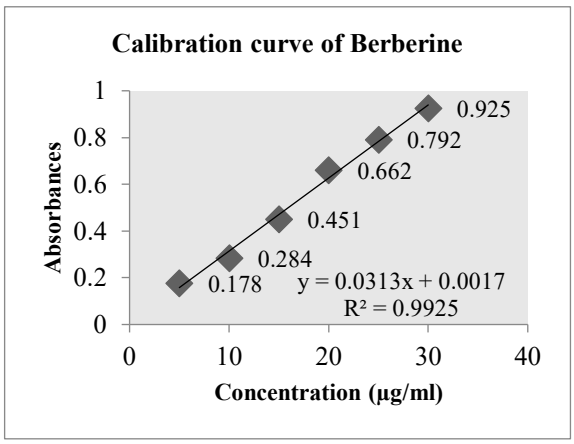

Figure 3: Calibration curve of Berberine

The absorbance versus concentration of Berberine in the 5-30 µg/ml range was plotted to create the regression equation. A calibration curve with six points was acquired for a drug concentration ranging from 5 to 30 µg/ml. The linear regression equation was y = 0.0313x + 0.0017 with correlation coefficient R2 = 0.9925, and the drug's response was determined being linear in the range of concentration under examination.

3.2.2 Functional group identified by Infra-Red spectroscopy

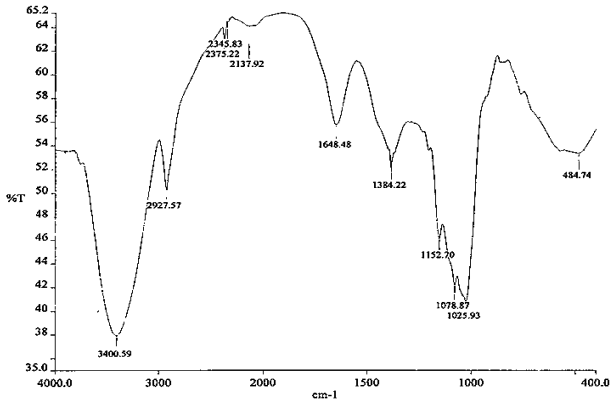

Figure 4: FTIR study of Berberine

Table 7: Interpretation of IR spectrum of Berberine

Peak obtained	Reference peak	Functional group	Name of functional group
3400.59	3400-3300	N-H stretching	Aliphatic primary amine
2927.57	3000-2840	C-H stretching	Alkane
2375.22	2400-2000 cm-1	O=C=O stretching	carbon dioxide
2137.92	2160-2120	N=N=N stretching	azide
1648.48	1690-1640	C=N stretching	Imine / oxime
1384.22	1372-1335	C-H bending	aldehyde
1152.70	1205-1124	C-O stretching	Tertiary alcohol
1025.93	1000-650 cm-1	C=C bending	alkene

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

Analysis of variance (ANOVA) is employed to ascertain the model's significance when in contrast to other models.

3.3.1 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**	13

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3.3.2 Formulation trials as per Box–Behnken design

Table 9 Formulation trials

Formulation Code	Beta- cyclo dextrin (Polymer) (mg) X1	Di-phenyl carbonate (DPC) (Cross linker) X2	Drug (mg)	Stirring time (Hrs) X3	Temperature (°C)	Distilled water (ml)	Particle size (nm) R1	Entrapment efficiency (%) R2
NSF 1	125	100	50	1	90 to 100	50	978.3	63.7
NSF 2	50	100	50	3.5	90 to 100	50	628.4	67.1
NSF 3	200	175	50	6	90 to 100	50	146.8	93.8
NSF 4	200	250	50	3.5	90 to 100	50	448.2	76.2
NSF 5	125	250	50	1	90 to 100	50	872.8	79.7
NSF 6	125	175	50	3.5	90 to 100	50	550.7	88.1
NSF 7	50	175	50	6	90 to 100	50	204.7	93.4
NSF 8	50	250	50	3.5	90 to 100	50	475.2	84.5
NSF 9	125	100	50	6	90 to 100	50	197.5	88
NSF 10	200	100	50	3.5	90 to 100	50	487	75.1
NSF 11	125	250	50	6	90 to 100	50	187.9	90.3
NSF 12	200	175	50	1	90 to 100	50	882.3	64.2
NSF 13	50	175	50	1	90 to 100	50	896.1	70.8

Table 10 Variables operating range for Nanosponges formulation

Name	Goal	Lower Limit	Upper Limit	Importance
A:Polymer	is in range	50	200	3
B:Cross linker	is in range	100	250	3
C:Stirring time	is in range	1	6	3
Particle size	none	146.8	978.3	3
Entrapment efficiency	none	63.7	93.8	3

3.3.4 Fit Summary

Table 11 Response 1: Particle size

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9835**	**0.9730**	**Suggested**

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2FI	0.2713	0.9865	0.9634	
Quadratic	0.2394	0.9922		
Cubic				Aliased

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

3.4.1 Response 1: Particle size

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

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	1.065E+06	3.550E+05	239.95	< 0.0001	significant
A-Polymer	7206.00	7206.00	4.87	0.0547	
B-Cross linker	11788.80	11788.80	7.97	0.0200	
C-Stirring time	1.046E+06	1.046E+06	707.01	< 0.0001	

The **Model F-value** of 239.95 implies the model is significant. There is only a 0.01% 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 B, C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Final equation in term of coded factor for Particle size

Particle size (R1) = +535.07 Intercept -30.01 X1 A-38.39 X2 B -361.58 X3 C. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

3.4.2 Predicted value and actual value of all formulations

Table 13: **Predicted value and actual value of all formulations**

Formulations	Observed result of Particle size	Predicted result of Particle size	Observed Results of % Entrapment efficiency	Predicted Results of % Entrapment efficiency
NSF 1	978.30	935.03	63.70	64.12
NSF 2	628.40	603.47	67.10	75.82
NSF 3	146.80	143.48	93.80	89.68
NSF 4	448.20	466.67	76.20	83.40
NSF 5	872.80	858.26	79.70	73.32
NSF 6	550.70	535.07	88.10	79.61
NSF 7	204.70	203.51	93.40	91.31

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NSF 8	475.20	526.69	84.50	85.02
NSF 9	197.50	211.88	88.00	85.90
NSF 10	487.00	543.44	75.10	74.20
NSF 11	187.90	135.11	90.30	95.10
NSF 12	882.30	866.63	64.20	67.91
NSF 13	896.10	926.66	70.80	69.53

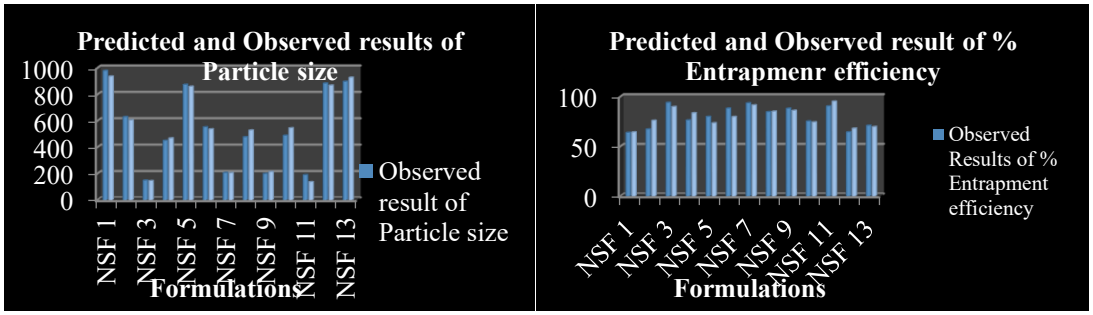

Figure 5: Graphical representation of Predicted and Observed results of Particle size, Figure 6: Graphical representation of Predicted and observed result of Entrapment efficiency

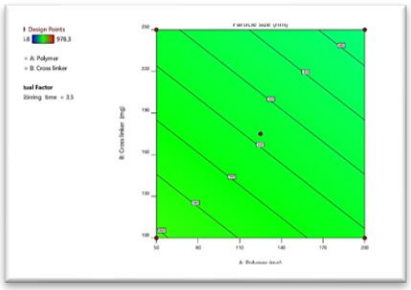

Figure 7: Two-dimensional (2D) contour plots for the effect of polymer and cross linker concentration on particle size

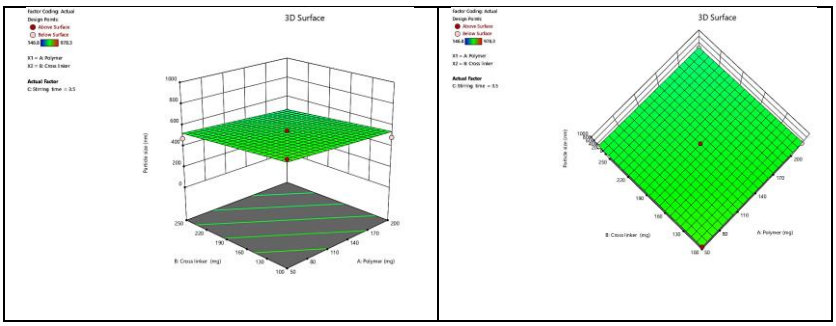

Figure 8: Response surface plot showing combined effect of Chemical on particle size of Nano sponges

3.4.3 Effect of formulation variables on Entrapment efficiency

Table 14 Response 2: Entrapment efficiency (Fit Summary)

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Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0.0023	0.9145	0.9795	Suggested
2FI	0.3332	0.7475	0.5173	
Quadratic	0.1754	0.8831		
Cubic				Aliased

3.5 ANOVA for Linear model

3.5.1 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	1122.86	374.29	11.01	0.0023	significant
A-Polymer	5.28	5.28	0.1553	0.7027	
B-Cross linker	169.28	169.28	4.98	0.0526	
C-Stirring time	948.30	948.30	27.89	0.0005	

The **Model F-value** of 11.01 implies the model is significant. There is only a 0.23% 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. Values greater than 0.1000 indicate the model terms are not significant

Final equation in Terms of Coded Factors for Entrapment efficiency

Entrapment efficiency (R2) = 79.61 Intercept -0.8125 X1 A +4.60 X2 B +10.89 X3 C. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

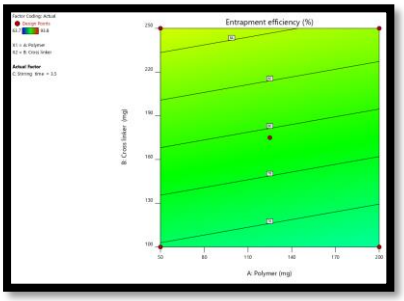

Figure 9: Two-dimensional contour plots for the effect of beta cyclodextrin and cross linker concentration on % entrapment efficiency

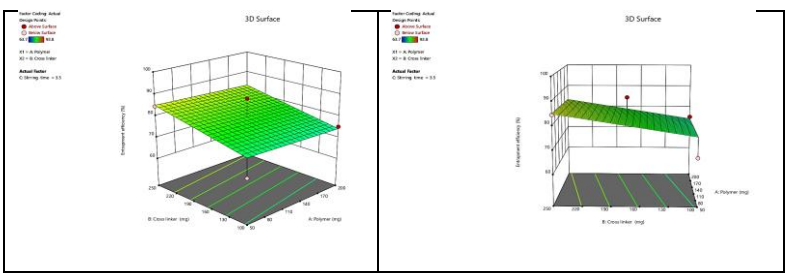

Figure10: Response surface plot showing combined effect of Polymer and cross linker on entrapment efficiency of Nano sponge's formulation (Three dimensional)

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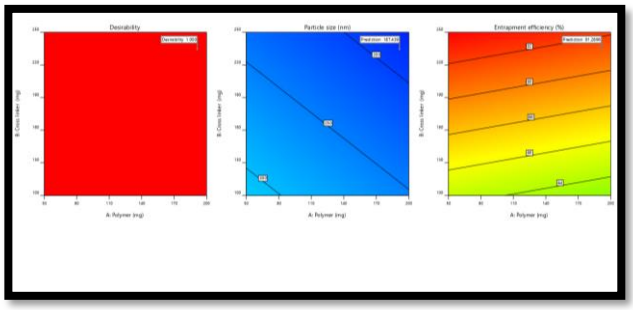

Figure 11: **Response surface plot showing prediction data for optimization**

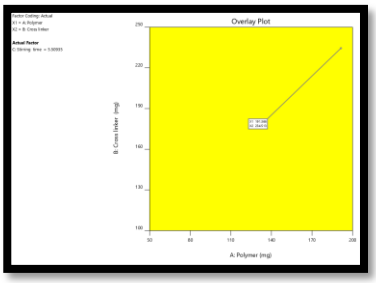

Figure 12: Overlay plot for optimization formulation

3.5.2 Optimized formula of Nanosponges formulation

Table 16: **Optimized formula of Nanosponges formulation**

S. No	Beta-cyclodextrin (Polymer)	DPC Cross linker (mg)	Stirring time (min.)	Particle size (nm)	Entrapment efficiency (%)	Desirability	
1.	**191.366**	**234.513**	**5.509**	**187.439**	**91.290**	**1.000**	**Selected**
2.	124.136	143.125	3.049	616.953	75.698	1.000	
3.	153.557	183.373	5.733	196.358	89.538	1.000	

Table 17: Final Composition of optimized Nano sponges formulation as per Design of experiment approach

S. No	Formulation Code	Beta- cyclo dextrin (Polymer) (mg) X1	Di-phenyl carbonate (DPC) (Cross linker) X2	Drug (mg)	Distilled water (ml)	Stirring time (Hrs) X3	Temperature (°C)
1	NSF	191.366	234.513	30	50	5.509	90-100 °C

3.6 Characterization of optimized formulation

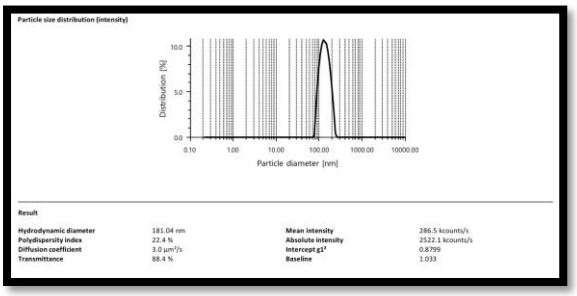

Figure 13: Particle size

Table 18: Particle size

S. No	Formulation	Particle size (Predicted Result)	Particle size (Observed results)
1.	Nanosponges	187.43	181.04 nm

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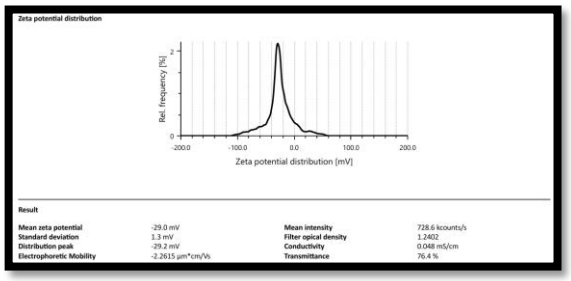

Figure 14: Zeta potential

Table 19: Zeta potential

S. No	Formulation	Zeta potential
1.	Nanosponges	- 29.0 mV

3.6.1 Entrapment efficacy

Table 20: Entrapment efficacy

S. No.	Formulations	Entrapment efficacy (Predicted result)	Entrapment efficacy (Observed result)
1.	Nanosponges	91.29 %	90.18 %

3.6.2 Scanning electron microscope (SEM)

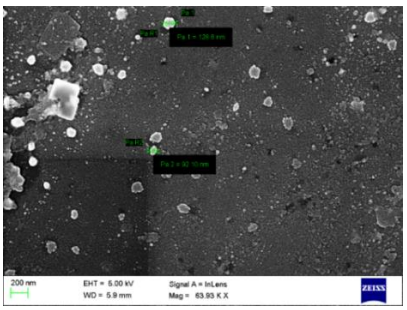

Figure 15: Scanning electron microscope (SEM)

A 63.93 kx magnification scanning electron micrograph of the created Nanosponges revealed it was spherical, smooth on the outside, and porous. The SEM pictures made it evident that the Nanosponges were porous.

3.6.3 *In-vitro* drug release

Table 21: 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
0	0	100	0.000	2.000	0.000	0.000
2	23.11	76.89	1.414	1.886	0.301	1.364
4	36.1	63.9	2.000	1.806	0.602	1.558
6	43.13	56.87	2.449	1.755	0.778	1.635
8	57.59	42.41	2.828	1.627	0.903	1.760
10	66.83	33.17	3.162	1.521	1.000	1.825
12	77.17	22.83	3.464	1.359	1.079	1.887
14	82.41	17.59	3.742	1.245	1.146	1.916
16	97.14	2.86	4.000	0.456	1.204	1.987

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3.6.4 Correlation value

Table 22: **Correlation value (R^2 value)**

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

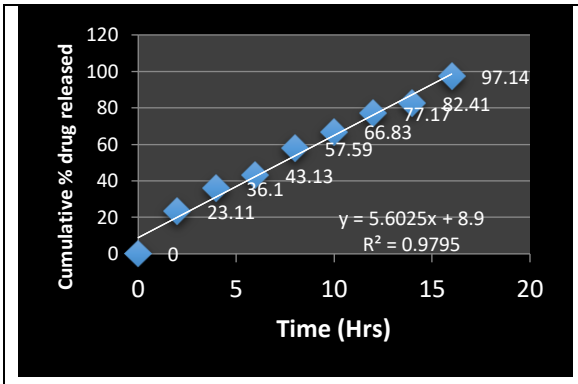

Figure 16: Zero order kinetic model

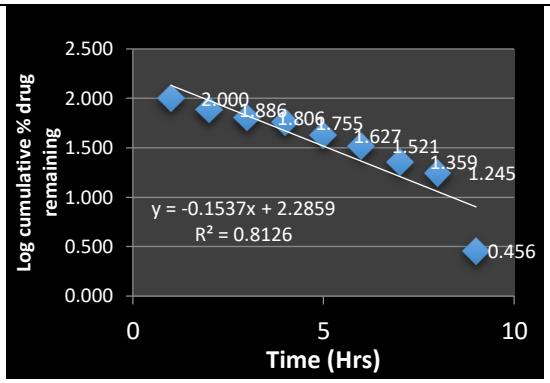

Figure 17: First Order kinetic model

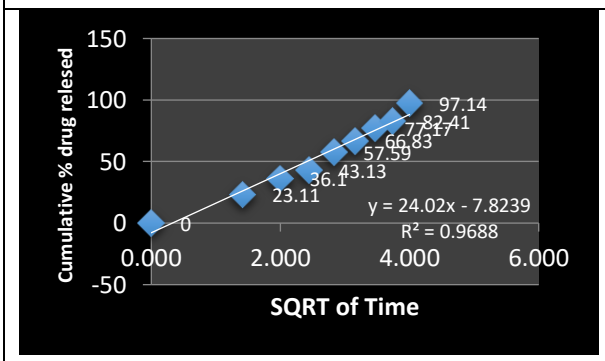

Figure 18: Higuchi model

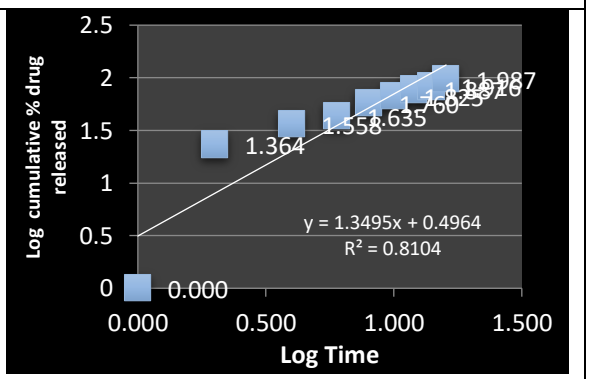

Figure 19: Korsmeyer peppas

3.7 Stability study

Table 23: Stability Study of optimized formulation (Nanosponges)

Time (Days)	25^{0}C±2 ^{0}C and 60 ± 5% RH		40^{0}C±2 ^{0}C and 70 ±5% RH	
	Particle Size (nm)	EE (%)	Particle Size (nm)	EE (%)
0	181.04 nm	90.18 %	181.04 nm	90.18 %
30	181.09 nm	90.11 %	181.09 nm	90.05 %

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45	180.90 nm	90.10 %	181.14 nm	90.01 %
60	181.01 nm	90.07 %	181.17 nm	90.03 %
90	180.95 nm	90.07 %	181.15 nm	90.00 %

Physically and chemically, the formulation was found to be stable for three months at accelerated stability conditions (25^0C±2 ^{0}C and 60±5% RH) and (40^0C±2 ^{0}C and 70±5% RH).

4. Conclusion

The study comes to the conclusion that the Design of Expert (DOE) approach, which is aided by Quality by Design (QbD), is a successful method for optimizing the manufacturing and formulation variables in the nanosponges containing Berberine. By determining and managing an ideal range of formulation and manufacturing variables, the QbD technique guarantees the quality of the final product. The predetermined product quality is ensured by this methodical approach to the design and development of pharmaceutical formulations and manufacturing procedures. The study shows that the number of trials needed to generate a cost-effective formula is decreased when QBD is applied in the optimization process. By incorporating quality into the process and product, DOE in pharmaceutical development lowers the possibility of mistakes and flaws. This study's future potential includes evaluating the effects of these approaches on the general quality and safety of the goods as well as applying QbD/DOE further in the creation of novel pharmaceutical formulations and manufacturing techniques.

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