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DESIGN, OPTIMIZATION AND EVALUATION OF OFLOXACIN LOADED NANOSPONGES IN GIT INFECTIONS USING BOX-BEHNKEN DESIGN

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Abstract

The objective of this study was to design ofloxacin loaded nanosponges which is a which is a BCS class II drug. Nanosponges are nano size particles which has reduced side effects as well as provide sustained release of drug. In the initial phase of the project, the pre- formulation studies were performed such as physical characterization, solubility of pure drug, determination of λ_{max} by UV spectroscopy, FT-IR of drug and solvents, along with calibration of drug. Further preparation of nanosponges were done by emulsion-solvent diffusion method utilising polyvinyl alcohol, dichloromethane, ethyl-cellulose, and drug- ofloxacin. Overall, fifteen formulations have been prepared with variation in drug concentration and solvents. The prepared nanosponges were subjected for evaluation for parameters such as particle size determination, % Drug Entrapment, % Drug Release, % Yield, FT-IR analysis, and SEM analysis of best formulation. The desired property of nanosponges was obtained by design of experiment method, according to which, the final optimized formulation obtained as A: 500mg, B: 100mg, C: 75mg. The particle size of optimized formulation was obtained as 289.9 nm, with 68 % drug release, 95.2% drug entrapment, and 88.2 % yield.

Keywords: Nanotechnology, Ofloxacin, Nanosponges, Emulsion Solvent Diffusion Method.

1. INTRODUCTION

In the recent scenario of drug delivery system, there is a crucial role of nano-technology in development of newer formulations for targeted, controlled, and sustained drug delivery system(1). Nano-technology has a great impact in the improvement of bio-availability of drug and the dosage form with plays an important role to lower the quantity of drug required to treat disease(2,3).

Nanosponges belongs to class of nano-technology which has nano sized particles helps to improve the bio-availability of the drug(4). The sustained release property of the formulation reduces the frequency of drug so that the administration of drug will not be required at regular interval of time(5)(1). With targeted delivery of drug, the drug will not be distributed to entire tissue, so it will reach to the desired part of the body to produce its effect(6).

Nanosponges are very small size particles of size 1 nm to 100 nm which are composed of different kinds of drugs(7). These formulated nanosponges can be further incorporated to form various kind of formulations like tablets, capsules, ointment, gel, lotions, creams, etc(8)(9)

Nanosponges has great advantage that it can be formulated as oral preparations, topical preparations, and parenteral preparations, so that it can provide delivery of drug through wide range of mechanism(9).

In this work, attempt has been made to evolve nanosponges loaded with ofloxacin, along with their evaluation for the treatment of gastro-intestinal infections with reduced quantity of drug, to overcome the adverse effect related to conventional dosage forms.

2. MATERIAL AND METHOD

2.1 Materials

Ofloxacin drug was a gift sample obtained from Bharat Pharmaceuticals. Ethyl cellulose, Dichloromethane, Polyvinyl alcohol was obtained from CDH private limited.

2.2 Solubility

The solubility analysis of ofloxacin was performed using phosphate buffer, 0.1N HCl, 0.1N NaOH, methanol ethanol. Methylene chloride, and water.

2.3 Preparation of calibration curve

A 100 mg of drug diluted in 0.1N HCl up to small amount. Further 100 ml volume made with using same 0.1N HCl. The above solution was referred as Stock I solution. In different flask, 10 ml above solution (Stock I) was diluted to prepare 100 ml Stock II solution using the same prepared 0.1N HCl.

Taking sample of 2 ml, 4 ml, 6 ml, 8 ml, 10 ml, serial dilutions were made from Stock II solution so as to produce desired concentration of 2 µg per ml, 4 µg per ml, 6 µg per ml, 8 µg per ml, 10 µg per ml. The absorbance of the different concentrations was evaluated at 294 nm by Ultra-violet spectrophotometer. A concentration vs absorbance graph was generated(10)

2.4 Preparatıon Of Ofloxacın Loaded Nanosponges

Formulation of ofloxacin loaded nanosponges was done using emulsion solvent diffusion method(11,12). Two phases were prepared in this method i.e, the aqueous phase as well as the organic phase.

Initially drug-ofloxacin and ethyl cellulose was mixed and diluted in dichloromethane of 20 ml to produce organic phase. Into another beaker, 100ml of distilled water was taken to which, desired quantity of polyvinyl alcohol was added to produce aqueous phase.

The aqueous phase was kept as magnetic stirrer at 1000 rpm for 1 hour with continuous mixing of organic phase for the formulation of nanosponges. The formulated product was filtered grade-1 Whatmann filter paper. The obtained product was dried under hot air oven at 40°**C** and then stored(13,14).

3. OPTIMIZATION OF OFLOXACIN LOADED NANOSPONGES BY DESIGN EXPERT (VERSION 12)

The design was implemented using Design-Expert® software (trial version 12, Stat-Ease), and a total of 15 runs were created(15). For the final optimization of ofloxacin loaded nanosponges, a surface response approach, Box-Behnken design with three level, three factor, was implemented. The drug and polymer ratio, were taken as independent factors. Whereas, % entrapment efficiency, % buoyancy and % Yield and Particle Size were considered as dependent responses(16).

Table 1: Factors in Box Behnken Design with their used levels

Factor	Name	Lower level (-1)	Upper level (+1)
A	Ethyl cellulose	200	500
B	PVA	100	500
C	Ofloxacin	50	100

A – Ethyl cellulose

B – PVA

C- Ofloxacin

Table 2: Summary by Design Expert (Version 12.0.3.0)

Study type	Response surfce
Design type	Box-Behnken
Sub Type	R andomized
Runs	15
Design Model	Quadratic

4. CHARACTERIZATION OF NANOSPONGES

4.1 Partıcle Sıze

The size of particles in each formulation were determined by DLS method i.e., dynamic light scattering for evaluation of particle size distribution.

4.2 Percentage Drug Entrapment-

In this method, required quantity of nanosponges were taken equivalent to quantity of drug. The nanosponges were dissolved in methanol and centrifuged for two hours. After completion, of centrifugation, 0.1ml of clear liquid was taken into volumetric flask of 10 ml, volume was made up with 0.1N HCl. By UV spectroscopy, absorbance was calculated at 294nm(17).

4.3 Percentage Drug Release:

Percentage drug release of all the formulations was calculated by determining the absorbance of sample.

4.4 Percentage Yıeld:

The percentage yield was measured by determining raw material initial weight and nanosponges final weight.

% yield= (Nanosponges weighed practically / Theoretical mass) X 100

5. RESULT & DISCUSSION

5.1 The particle size, % Drug Entrapment, % Drug Release, and % Yield of each formulation was determined and the observed date for analysis in given in the table below:

Table 3: Box–Behnken Design- Result table of ofloxacin loaded Nanosponges

Std	RUN	Factor-1 A: Ethyl Cellulose (mg)	Ethy Factor-2 B: PVA (mg)	Factor-3 C: Ofloxacin (mg)	Response 1 Particle size (nm)	Response2 % Drug Entrapment	Response 3 % Drug Release	Response 4 % Yield
7	3	200	300	100	250.33	250.33	74	95
1	6	200	100	75	256.13	256.13	80	93
3	7	200	500	75	259.22	259.22	82	94
5	12	200	300	50	260.11	260.11	84	83
10	2	350	500	50	261.23	261.23	87	84
12	4	350	500	100	273.2	273.2	88	76
11	8	350	100	100	278.15	278.15	89	75
9	10	350	100	50	281.5	281.5	90	75
15	11	350	300	75	285.12	285.12	91.2	73
14	13	350	300	75	286.12	286.12	93	71
13	14	350	300	75	287.4	287.4	94.31	70
2	1	500	100	75	289.9	289.9	95.2	68
8	5	500	300	100	290.3	290.3	97.6	68
6	9	500	300	50	294.4	294.4	98.2	66
4	15	500	500	75	297.32	297.32	99.2	65

5.2 Solubility:

The excellent solubility of ofloxacin was found in 0.1N HCl.

5.3 Particle Size Determınation:

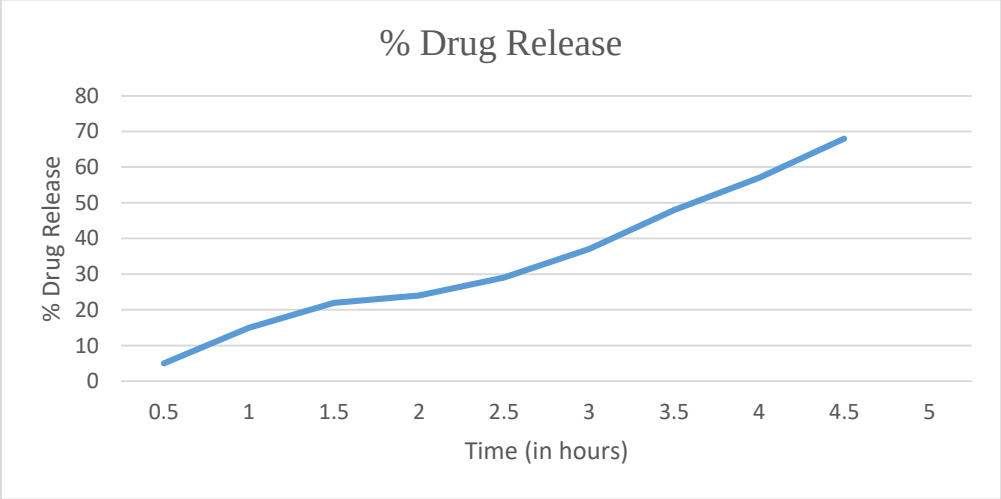
The average particle size of all the formulation was measured and obtained between 250.23 nm to 297.32 nm. The particle size of final optimized formulation was obtained as 289.9 nm.

5.4 % Drug Entrapment:

The entrapment efficiency of all the formulation was found between 74% to 98.9%. The % drug entrapment of final optimized formulation was found to be 95.2 %.

5.5 % Drug Release:

The percentage drug release was measured between 65% to 95%. % drug release of optimized formulation was obtained as 68%.



Graph: Showing İn-Vitro Release Of Optimized Formulation Of Ofloxacin Loaded Nanosponges

5.6 % Yield:

The percentage yield for all the formulation was obtained between 70% to 93%. The % yield of final optimized formulation was obtained as 88.2%.

5.7 Model Analysıs

5.7.1 ANOVA - Quadratic Model

Response1- Particle Size

Table 4: ANOVA - Quadratic model

Source	Squares Sum	df	Mean-Square	F-value	p-value	
Model	3126.3	9	347.36	7.66	0.0187	Significant
A-Ethyl Celluslose	2672.9	1	2672.9	58.92	0.0006	
B-PVA	27.05	1	27.05	0.5963	0.4749	
C-Offloxacin	3.59	1	3.59	0.0792	0.7897	
AB	4.69	1	4.69	0.1033	0.7609	
AC	8.35	1	8.35	0.1841	0.6857	
BC	58.68	1	58.68	1.29	0.307	
A^2	98.52	1	98.52	2.17	0.2006	
B^2	107.88	1	107.88	2.38	0.1837	
C^2	196.11	1	196.11	4.32	0.0922	
Residual	226.81	5	45.36			
Lack of Fit	224.2	3	74.73	57.22	0.0172	Not Significant
Pure Error	2.61	2	1.31			
Cor Total	3353.1	14				

Table 5: Fit Statistics

Fit Statistics			
Std. Dev.	6.74	R^2	0.9324
Mean	276.69	Predicted R^2	-0.072
C.V. %	2.43	Adjusted R^2	0.8106
		Adeq Precision	7.5122

Final Equation in Terms of Coded Factors

Particle Size	= +286.21+18.28A-1.84B-0.6700C+1.08AB+1.45AC+3.83BC-5.17A^2-5.41B^2-7.29C^2

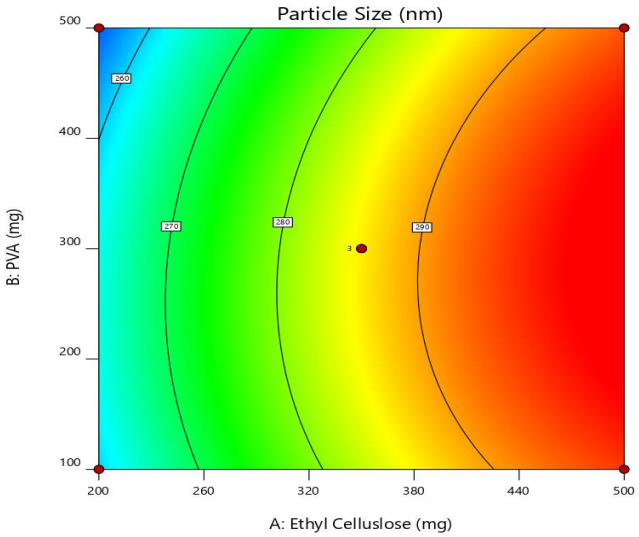
The coded factors equation may be implemented for making suggestion concern to response of each factor for the given levels. By default, factors with large level are coded as +1 along with factors with lower level as -1. The equation in coded makes functional for identification of factors comparative impact by differentiating factor coefficients.

Factor Coding: Actual

Particle Size (nm)
Design Points

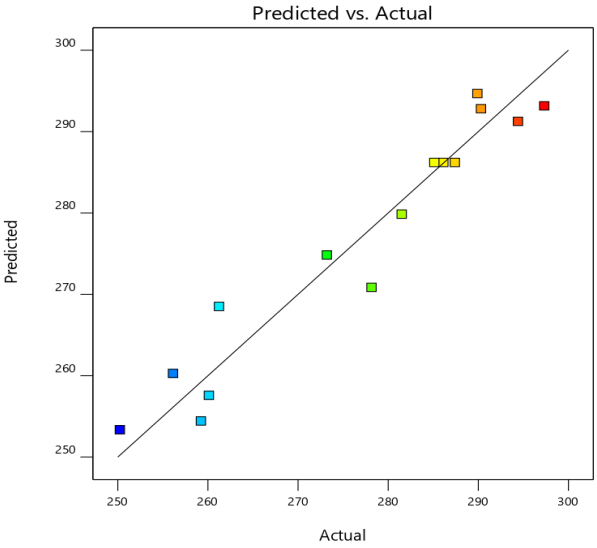
250.23 297.32
X1 = A: Ethyl Cellulslose
X2 = B: PVA

Actual Factor
C: Offloxacin = 75



Particle Size

Color points by value of
Particle Size:
250.23 297.32



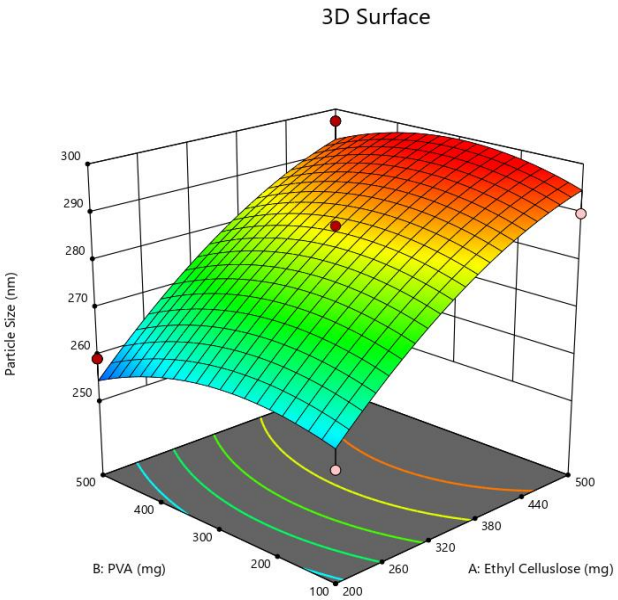
Factor Coding: Actual

Particle Size (nm)
Design Points:
Above Surface
Below Surface
250.23 297.32

Actual Factor
C: Offloxacin = 75

X1 = A: Ethyl Cellulslose
X2 = B: PVA

Actual Factor
C: Offloxacin = 75



A **R^2 Predicted** negatively suggested that, as compared to current model, the overall mean can be greater for prediction of the response. In few cases, it is better to imply higher order model for better prediction.

Adeq Precision measures the signal in the form of noise ratio. The recommended ratio should be above 4. The obtained ratio is 7.512 indicating sufficient signal. The model can be implemented This model may be utilised steer the design space.

Factor-coding was **Coded**. Squares Sum is **Type III- Partial**

The **F-value in the model is** 7.66 indicates that particular model is important. Only 1.87% chance that because of noise, the large F-value could occur.

P-value below 0.0500 implies that model term is important. A is an important model term. Readings above 0.1000 suggests that the model term is not significant. In case of larger insignificant model terms, model reduction makes the model better.

Obtained **F-value of Lack of Fit** is 57.22 suggest that significant Lack of Fit. Only 1.72% possibility that F-value of Lack of Fit. This large F-value caused by noise could occur. Lack of fit significant is bad -desired fit model.

5.7.2 ANOVA - Quadratic model

Response 2 - Drug Entrapment

Table 6: ANOVA - Quadratic model

Source	Squares Sum	df	Mean-Square	F-value	P-value	
Model	691.68	9	76.85	11.06	0.0083	significant
A-Ethyl Celluslose	610.75	1	610.75	87.89	0.0002	
B-PVA	0.3612	1	0.3612	0.0520	0.8287	
C-Offloxacin	14.04	1	14.04	2.02	0.2144	
AB	0.7225	1	0.7225	0.1040	0.7602	
AC	22.09	1	22.09	3.18	0.1347	
BC	1.0000	1	1.0000	0.1439	0.7200	
A^2	13.77	1	13.77	1.98	0.2183	
B^2	13.06	1	13.06	1.88	0.2287	
C^2	22.27	1	22.27	3.20	0.1334	
Residual	34.74	5	6.95			
Lack of Fit	29.87	3	9.96	4.08	0.2029	Not significant
Pure error	4.88	2	2.44			
Cor Total	726.43	14				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **model F-value** of 11.06 indicates that particular model is important. Only 1.87% chance that because of noise, the large F-value could occur.

P-value below 0.0500 implies that model term is important. A is an important model term. Readings above 0.1000 suggests that the model term is not significant. In case of larger insignificant model terms, model reduction makes the model better.

Obtained **F-value of Lack of Fit** is 4.02 suggest the not significant Lack of Fit to the pure error. Only 20.09% chance that a F-value Lack of Fit this large F-value caused by noise could occur. Lack of fit significant is bad - desired fit model.

Table 7: Fit Statistics

Fit Statistics			
Std. Dev.	2.64	R^2	0.9522
Mean	89.49	Predicted R^2	0.3270
C.V. %	2.95	Adjusted R^2	0.8661
		Adeq Precision	10.3900

5.7.3 ANOVA for Quadratic model

Response 3: Drug Release

Table 8: ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1395.27	9	155.03	8.10	0.0165	significant
A-Ethyl Celluslose	1200.50	1	1200.50	62.74	0.0005	
B-PVA	8.00	1	8.00	0.4181	0.5464	
C-Offloxacin	4.50	1	4.50	0.2352	0.6482	
AB	4.00	1	4.00	0.2091	0.6667	
AC	25.00	1	25.00	1.31	0.3048	
BC	16.00	1	16.00	0.8362	0.4024	
A^2	77.56	1	77.56	4.05	0.1002	
B^2	61.56	1	61.56	3.22	0.1328	
C^2	16.03	1	16.03	0.8376	0.4021	
Residual	95.67	5	19.13			
Lack of Fit	91.00	3	30.33	13.00	0.0723	not significant
Pure Error	4.67	2	2.33			
Cor Total	1490.93	14				

5.7.4 ANOVA for Quadratic model

Response 4: Percentage yield

Table 9: ANOVA - Quadratic model

Source	Squares Sum	df	Mean-Square	F-value	p-value	
Model	655.60	9	72.84	7.38	0.0202	significant
A-Ethyl Celluslose	556.11	1	556.11	56.38	0.0007	
B-PVA	0.1250	1	0.1250	0.0127	0.9147	
C-Offloxacin	4.96	1	4.96	0.5030	0.5099	
AB	0.0625	1	0.0625	0.0063	0.9396	
AC	6.76	1	6.76	0.6853	0.4455	
BC	2.72	1	2.72	0.2760	0.6218	
A^2	26.75	1	26.75	2.71	0.1605	
B^2	24.32	1	24.32	2.47	0.1771	
C^2	46.31	1	46.31	4.70	0.0825	
Residual	49.32	5	9.86			
Lack of Fit	48.71	3	16.24	53.53	0.0184	Not significant
Pure Error	0.6067	2	0.3033			
Cor Total	704.92	14				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **model F-value** of 7.38 indicates that particular model is important. Only 2.02% chance that caused noise, the large F-value could occur.

P-value below 0.0500 implies that model term is important. A is an important model term. Readings above 0.1000 suggests that the model term is not significant. In case of larger insignificant model terms, model reduction makes the model better.

Obtained **F-value of Lack of Fit** is 53.53 suggest that significant Lack of Fit. Only 1.84% chance that a F-value Lack of Fit this large F-value caused by noise could occur. Lack of fit significant was bad - desired fit model.

Table 10: Fit Statistics

Fit Statistics			
Std. Dev.	3.14	R^2	0.9300
Mean	82.94	Adjusted R^2	0.8041
C.V. %	3.79	Predicted R^2	-0.1076
		Adeq Precision	7.6969

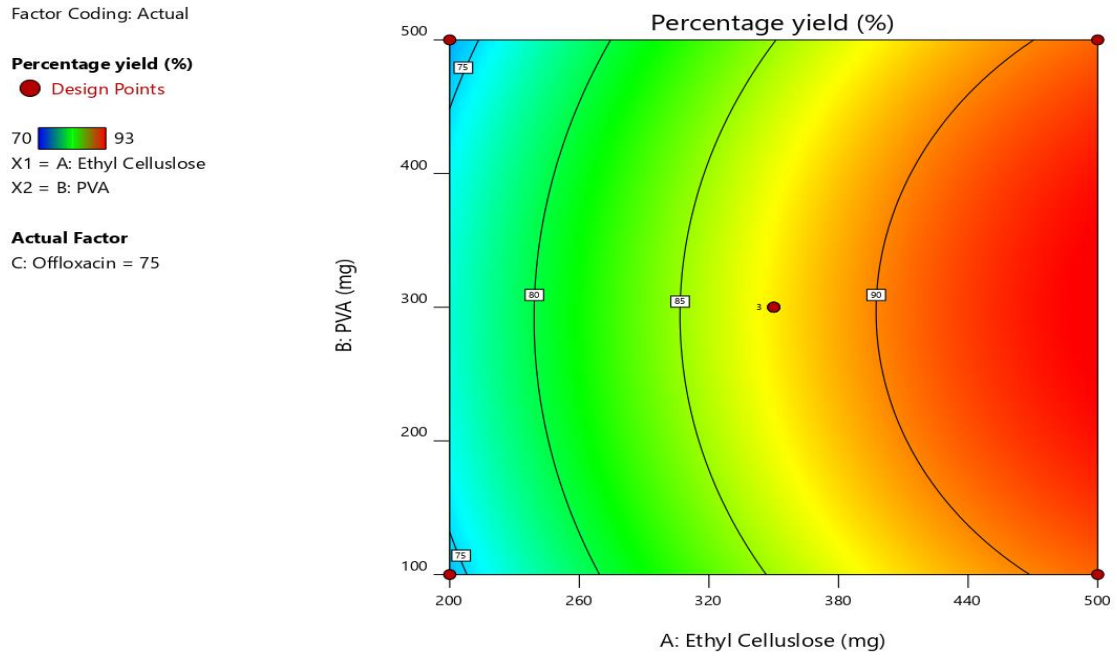
A **R^2 Predicted** negatively suggested that, as compared to current model, the overall mean can be greater for prediction of the response. In few cases, it is better to imply higher order model for better prediction.

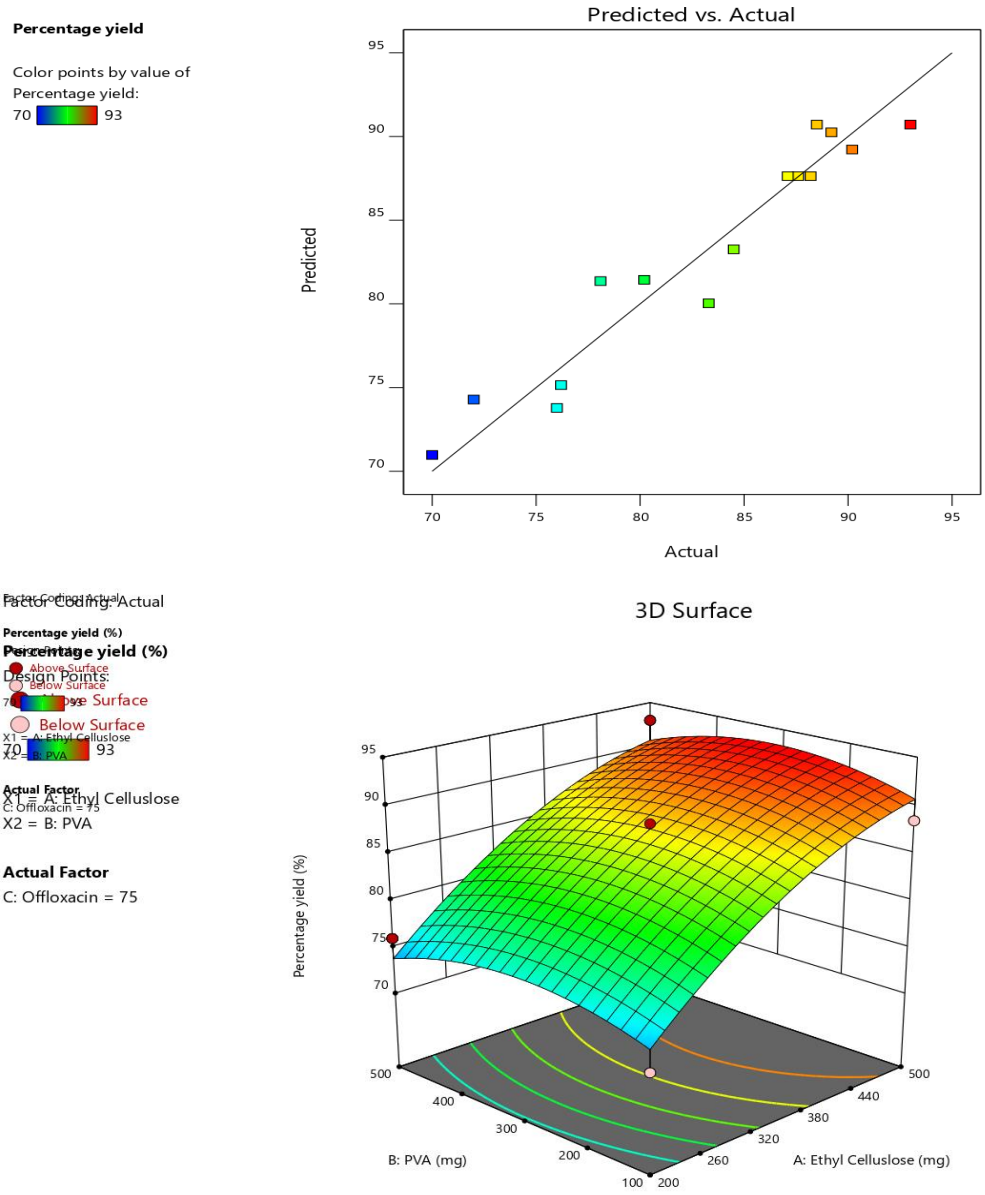
Adeq Precision measures the signal in the form of noise ratio. The recommended ratio should be above 4. The obtained ratio is 7.697 indicating sufficient signal. The model can be implemented This model may be utilised steer the design space.

Final Equation in Terms of Coded-Factors

Percentage yield	$=+87.63+8.34A-0.1250-0.7875C+0.1250AB+1.30AC+0.8250BC-2.69A^2-2.57B^2-3.54C^2$

The coded factors equation may be implemented for making suggestion concern to response of each factor for the given levels. By default, factors with large level are coded as +1 along with factors with lower level as -1. The equation in coded form makes functional for identification of factors comparative impact by differentiating factor coefficients.





Factor-coding is **Coded**.

Sum of squares is **Type II- Partial**

The **model F-value** of 8.10 indicates that particular model is important. Only 1.65 % chance that caused by noise, the large F-value could occur.

P-value below 0.0500 implies that model term is important. A is an important model term. Readings above 0.1000 suggests that the model term is not significant. In case of larger insignificant model terms, model reduction makes your model better.

The **F-value of Lack of Fit** is 13.00 suggest that significant Lack of Fit. Only 7.23% chance that a F-value Lack of Fit this large F-value caused by noise could occur. Lack of fit significant is bad -desired fit model.

Table 11: Fit Statistics

Fit Statistics			
Std. Dev.	4.37	R^2	0.9358
Mean	77.07	Predicted R^2	0.0164
C.V. %	5.68	Adjusted R^2	0.8203
		Adeq Precision	8.4698

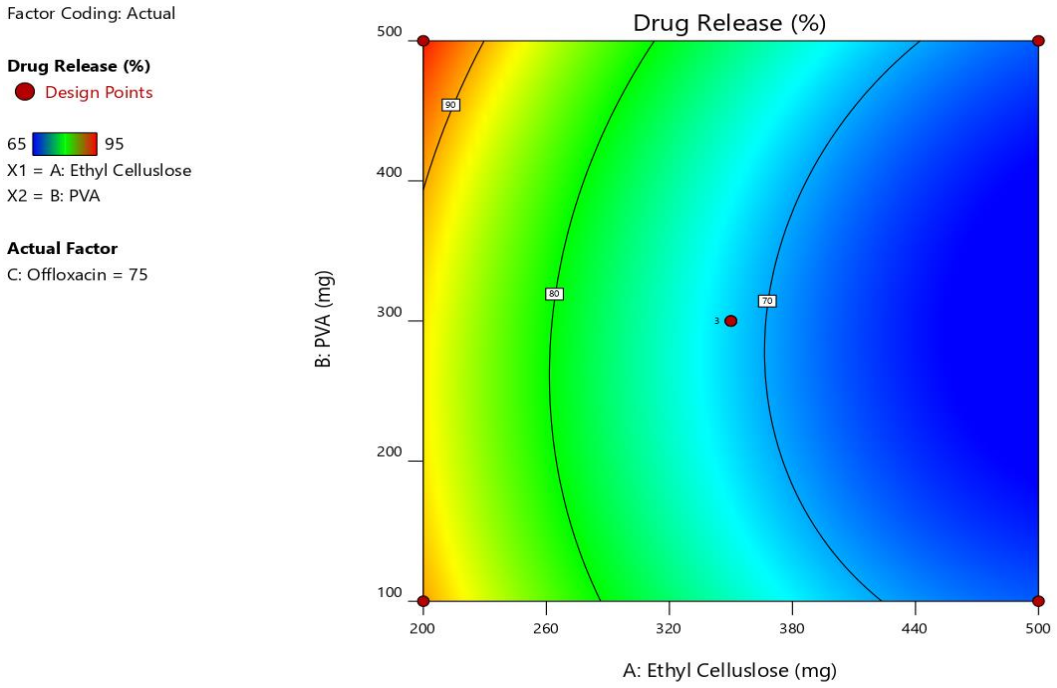
The **R^2 Predicted** 0.0164 is not as closer to the **R^2 Adjusted** 0.8203 so, the difference obtained is more than 0.2. It suggests that there may be a feasible problem as data or model. Points that may be considered, response transformation, model reduction, outliers, etc. Every empirical model must be evaluated by confirmation runs.

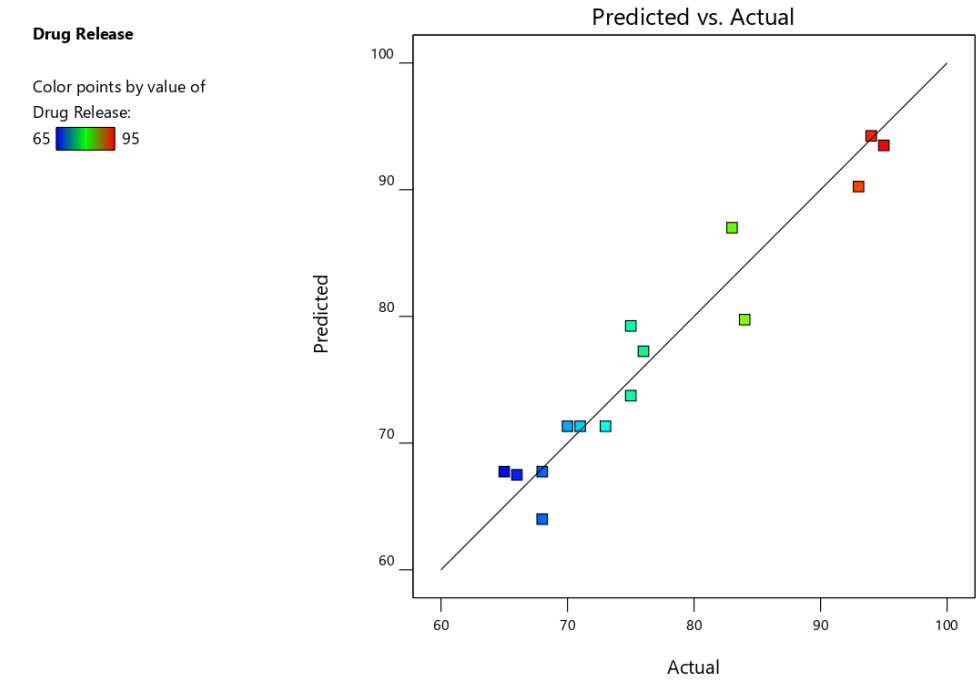
Adeq Precision measures the signal in the form of noise ratio. The recommended ratio should be above 4. The obtained ratio is 8.470 indicating sufficient signal. The model can be implemented This model may be utilised steer the design space.

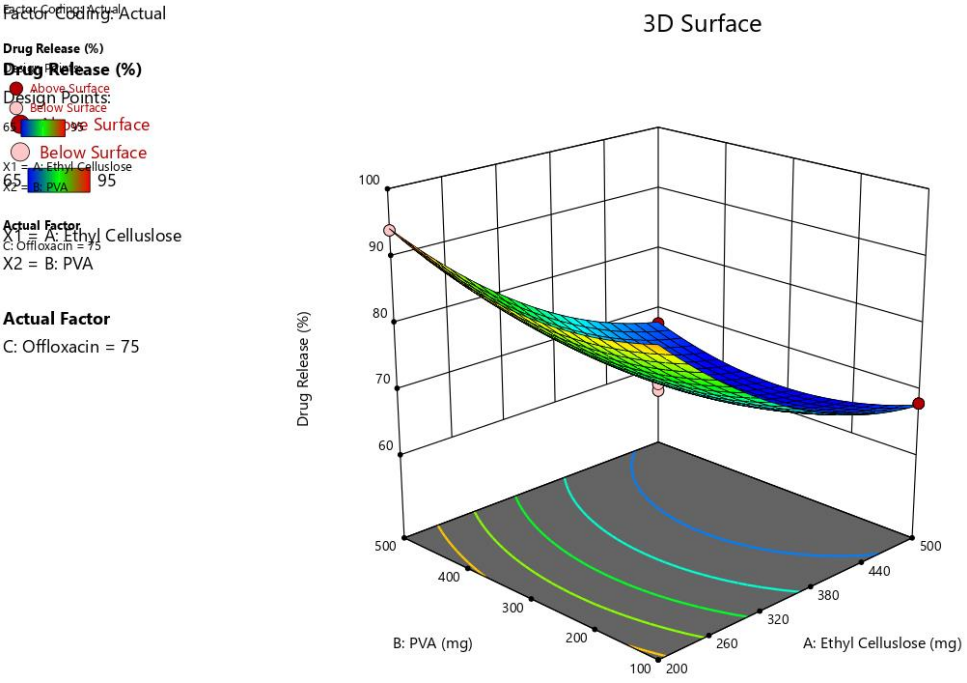
Final Equation in Terms of Coded Factors

Drug Release	$=+71.33-12.25A+1.0000B+0.7500C-1.0000AB-2.50AC-2.00BC+4.58A^2+4.08B^2+2.O8C^2$

The coded factors equation may be implemented for making suggestion concern to response of each factor for the given levels. By default, factors with large level are coded as +1 along with factors with lower level as -1. The equation in coded form makes functional for identification of factors comparative impact by differentiating factor coefficients.







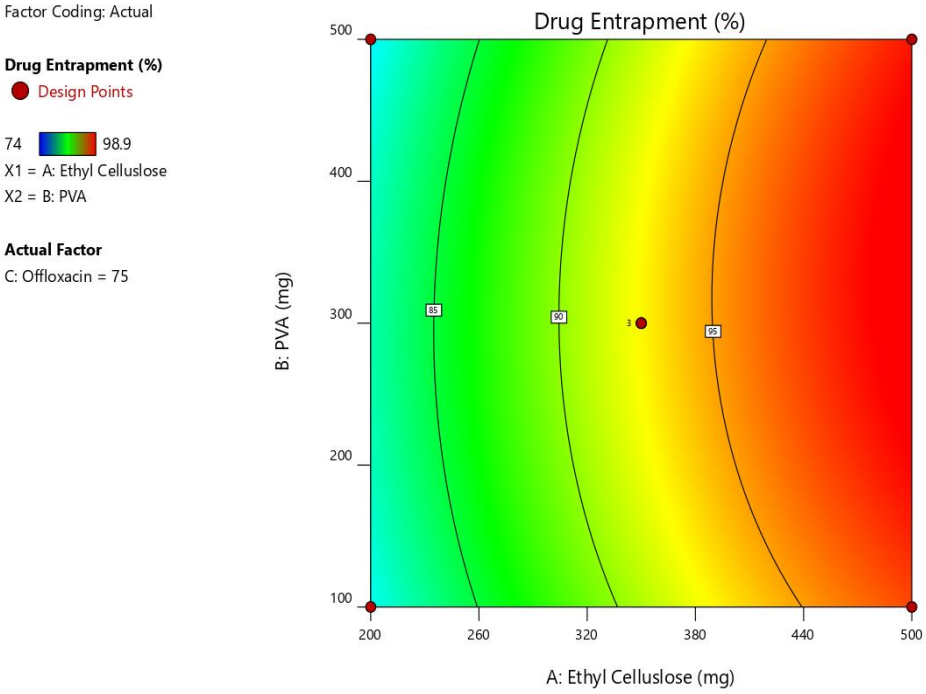
The **R² Predicted** 0.3270 is not as closer to the **R² Adjusted** 0.8661 so, the difference obtained is more than 0.2. It suggests that there may be a feasible problem as data or model. Points that may be considered, response transformation, model reduction, outliers, etc. Every empirical model must be evaluated by confirmation runs.

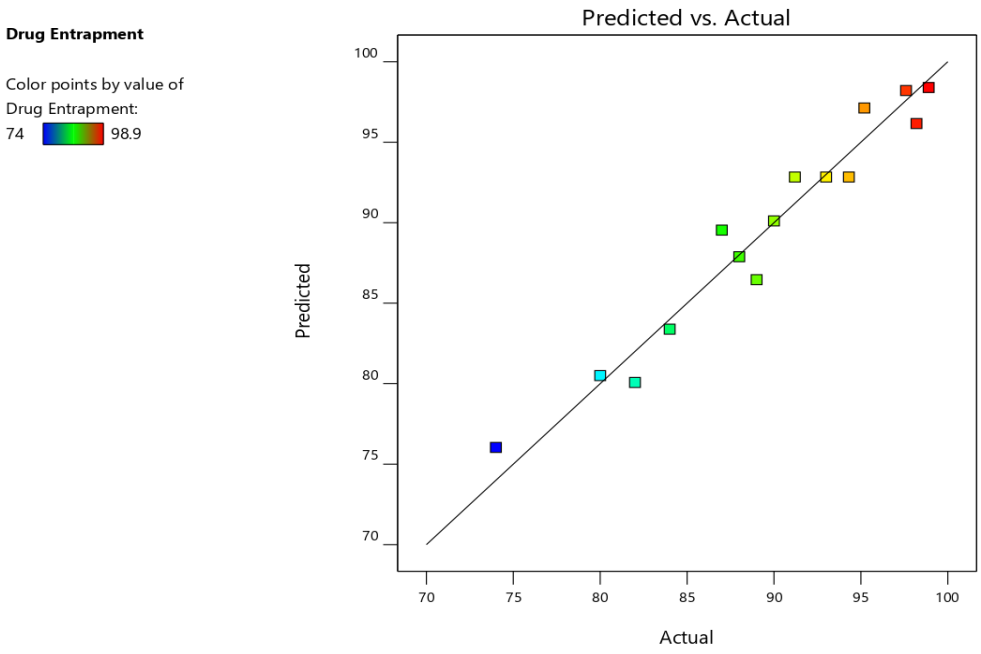
Adeq Precision measures the signal in the form of noise ratio. The recommended ratio should be above 4. The obtained ratio is 10.390 indicating sufficient signal. The model can be implemented This model may be utilised steer the design space.

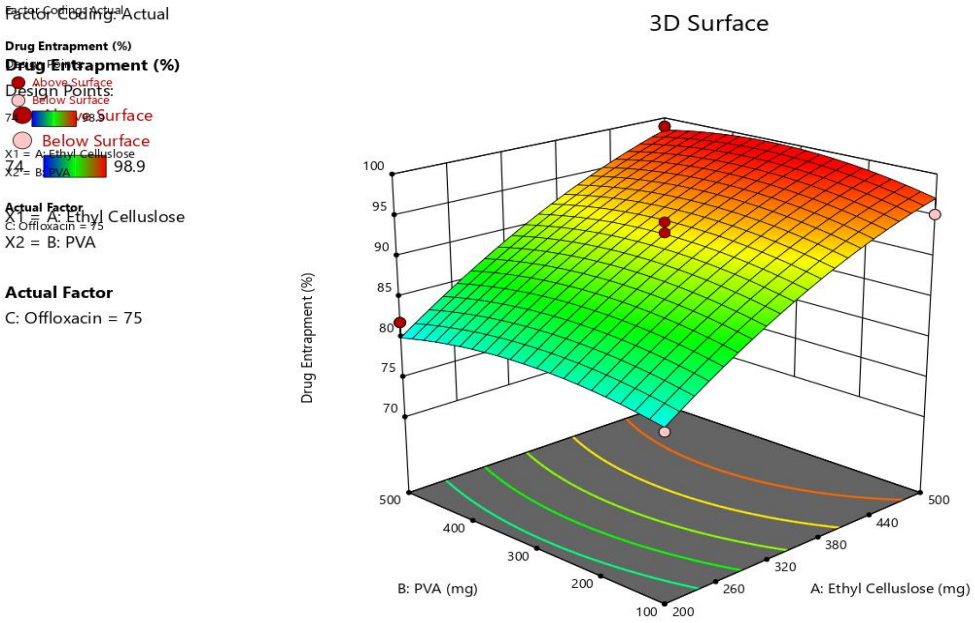
Final Equation in Terms of Coded Factors

Drug Entrapment	= +92.84+8.74A+0.2125B-1.33C+0.4250AB+2.35AC+0.5000BC-1.93A^2-1.88B^2-2.46C^2

The coded factors equation may be implemented for making suggestion concern to response of each factor for the given levels. By default, factors with large level are coded as +1 along with factors with lower level as -1. The equation in coded form makes functional for identification of factors comparative impact by differentiating factor coefficients.







Zeta Potentıal:

The figure potential of optimized nanosponges were obtained as -22.85 mV with intensity of 98.4%.

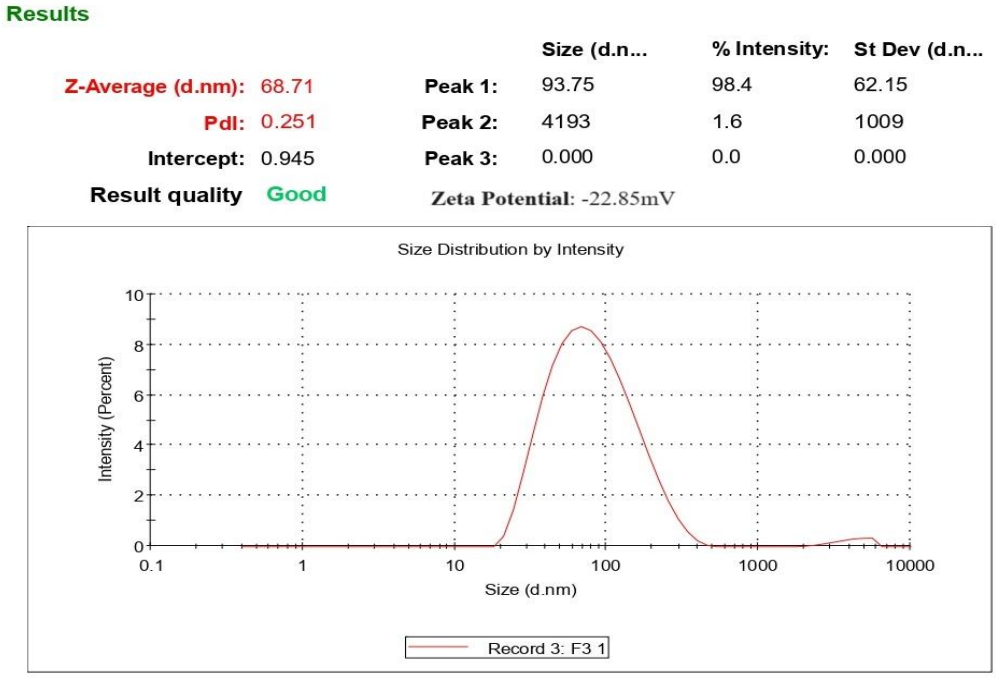


Figure: Figure showing Zeta Potential and intensity of nanosponges final formulation

Morphology And Surface Topography:

The morphological properties of ofloxacin loaded nanosponges were studied by SEM analysis and evaluated. The shape of nanosponges were examined as spherical shaped. The droplet size observed between the range of 10µm to 100µm and distributed evenly in nanometre range. The observed image was shown in the figure-

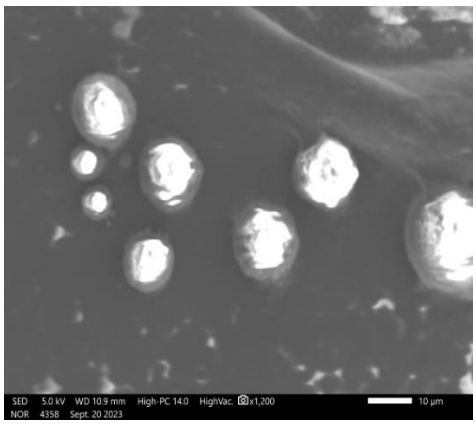


Figure: Figure showing SEM evaluation of nanosponges

6. CONCLUSION

Nanosponges loaded with ofloxacin was successfully formulated to enhance the sustained and controlled drug release. Implementation of experimental design was found to be impactful tool for the development of ofloxacin loaded nanosponges. The nanosponges preparation was carried out using emulsion solvent diffusion method, in which ethyl cellulose was dissolved in organic solvent and polyvinyl alcohol was dissolved in aqueous solvent. Using three different factors as ofloxacin, ethyl cellulose, and polyvinyl alcohol, different formulations were prepared. These formulations were studied with four responses as particle size determination, % drug release, % entrapment and % yield. Box-Behnken design was improvised to get desired optimized formulation.

In all formulations, final optimized formulation was obtained by using experimental design method. The morphological analysis of final optimized formulation was done by SEM method. The optimized formulation particle size was obtained as 289.9 nm, with 68 % drug release, 95.2% drug entrapment, and 88.2 % yield.

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