Available online on 15.11.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Check for updates

Research Article

Screening the statistical impact of some independent variables on the particle size and entrapment efficiency (EE) for the design and development of curcumin-loaded PLGA-Tf nanoparticles

Deepika Verma[1*], Kumud Upadhyaya[2]

[1] *Ph.D Scholar, Department of Pharmacy Kumaun University Nainital Bhimtal, Uttarakhand, India*

[2] *Professor, Department of Pharmacy Kumaun University Nainital Bhimtal, Uttarakhand, India*

Article Info:

Article History:

Received 11 Aug 2023
Reviewed 03 Oct 2023
Accepted 25 Oct 2023
Published 15 Nov 2023

Cite this article as:

Verma D, Upadhyaya K, Screening the statistical impact of some independent variables on the particle size and entrapment efficiency (EE) for the design and development of curcumin-loaded PLGA-Tf nanoparticles, Journal of Drug Delivery and Therapeutics. 2023; 13(11):13-20

DOI: http://dx.doi.org/10.22270/jddt.v13i11.6279

*Address for Correspondence:

Deepika Verma, Ph.D Scholar, Department of Pharmacy Kumaun University Nainital Bhimtal, Uttarakhand, India

Abstract

The present study provides the insight of statistical aspects of various independent variables like Tf-PLGA Curcumin ratio, stirring speed and emulsifying concentration over dependent variables like particle size and EE of the drug. Design Expert Version 13.1.5 was used to analyse the impact of independent variables over dependent variables. To study the effect of intendent variables over particle size, the valve Predicted R^2 was 0.9701 is in reasonable agreement with the Adjusted R^2 of 0.9907; i.e., the difference is less than 0.2. indicating that stirring speed have a great impact of particle size. While in another fact also shown the Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable and in respect of that the ratio of 9.630 indicates an adequate signal, i.e. it greatly gives impact on size of nanoparticles. In the case of entrapment efficacy (EE) the Predicted R^2 of 0.9892 is in reasonable agreement with the Adjusted R^2 of 0.9910; i.e. the difference is less than 0.2 which support the significance of the study and impact of Tf- PLGA curcumin ratio over EE. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable but for this study the ratio was calculated as14.989 indicates an adequate signal. Therefore, the two independent variables like, Tf-PLGA Curcumin ratio and stirring speed have significant impact on EE and particle size of nanoparticles.

Keywords: Tf-PLGA Curcumin ratio, stirring speed, EE, particle size, Adjusted R^2, Predicted R^2,. Adeq Precision

INTRODUCTION

Nanotechnology is a cutting-edge field of science and technology that deals with the manipulation and control of matter at the nanoscale, typically at dimensions of 1 to 100 nanometres[1]. At this incredibly small scale, the properties and behaviours of materials can differ significantly from their macroscale counterparts. Nanotechnology encompasses a wide range of disciplines, including physics, chemistry, biology, engineering, and materials science[2]. Pharmaceutical polymers play a pivotal role in the design and development of various dosage forms, contributing to the safety, efficacy, and patient acceptability of pharmaceutical products[3-4]. The effect of curcumin-loaded PLGA-Tf (Transferrin-Modified Poly (lactic-co-glycolic acid)) nanoparticles on particle size and encapsulation efficiency has been a subject of significant research interest due to its potential implications for drug delivery applications. Several studies have explored this topic, shedding light on the relationship between formulation parameters and these critical nanoparticle characteristics[5-6]. Using Design of Experiments (DoE) software version 13.1.5, the current study investigated the statistical effects of independent variables such as curcumin-loaded PLGA-Tf nanoparticles, stirring speed, and surfactant (span 80) on dependent variables such as entrapment efficiency (EE) and particle size of curcumin-loaded nanoparticles. The effect of independent factors over dependent variables has been clearly demonstrated by the study. The dependent variables have received substantial weight in the statistical data[7].

MATERIALS AND METHOD

Chemicals, such as PLGA, transferrin, and Span 80, were gifts from Varav Biogenesis Ltd., Kala-amb, H.P., India. Pure curcumin was purchased on the open market. The remaining chemicals and reagents were of the laboratory variety.

Method of Preparation of curcumin-loaded PLGA-Tf nanoparticles

Microemulsion precursor method

The microemulsion precursor method was used for the preparation of nanoparticles. It involved the use of a microemulsion system, which was a thermodynamically stable dispersion of two immiscible liquids. The oil phase was typically a nonpolar solvent such as ethyl oleate, while the surfactant (Span 80) and cosurfactant (PEG 400) were chosen based on their ability to form a stable microemulsion. Dissolved curcumin in the oil phase of the microemulsion. This involved the PLGA polymer and the organic solvent. Further, transferrin was added to the oil phase, allowing it to become incorporated within the microemulsion. Gradually added the microemulsion to the aqueous phase while stirring gently. This forms an oil-in-water microemulsion droplet system. The surfactants at the interface of the microemulsion droplets encapsulated the curcumin-loaded PLGA-transferrin conjugates as the microemulsion was added to the aqueous phase. The cross-linking agent 1% glutaraldehyde was introduced to enhance nanoparticle stability. Centrifuge the microemulsion mixture to isolate the nanoparticles formed during the emulsification process. Wash the nanoparticles to remove excess surfactants, solvents, and any unreacted components[8].

Optimization of the Formulation Process

The formulation of curcumin-loaded Tf-conjugated PLGA nanoparticles can be optimised by using Design of Experiments (DoE) verion 13.1.5., notably the Box-Behnken design with three components and two levels. Particle size (Y1) and the percentage of medication encapsulated (Y2) allow for a systematic examination of the effects of two independent factors on two critical responses. It can effectively explore the experimental space, find ideal circumstances, and build polynomial models by using DoE and Design Expert® software. After measuring the responses with either simple linear (Y = X0 + X1A + X2B), interactive (Y = X0 + X1A + X2B + X5AB), or quadratic (Y = X0 + X1A + X2B + X3A2 + X4B2 + X5AB + E) models, the values of selected variables at different levels can be obtained by multiple regression analysis of the data and F statistics to identify the statistically significant terms. Then, in order to examine the impact of particular variables while switching from low to high level, contour plots are created using the reduced equation (i.e., an equation based exclusively on statistically significant terms). The non-linear quadratic model produced by the design has the formula Y = X0 + X1A + X2B + X3A2 + X4B2 + X5AB + E, where Y is the measured response connected to each combination of factor levels: A and B are the factors under investigation, X0 is an intercept, X1 through X5 are the regression coefficients, and E is the related error term.

On the basis of the findings of the pre-optimisation research, three square 2 level Box-Behnken design were chosen for optimisation. The PLGA-Tf-Curcumin concentration, stirring speed and surfactant concentration were shown to have a substantial impact on particle size and encapsulation efficiency. The performance of the formulation depends heavily on the efficacy of the encapsulation and particle size, hence these responses were chosen[9].

Table.1, displays the independent concentrations and their range, which means they can be used. Particle size and entrapment effectiveness responses were statistically assessed and are displayed in Table No.2

Table 1: Ranges of the Factors Investigated Using Box–Behnken 3 factor ,2 level Experimental Design for nanoparticle of *curcumin*

Independent Factors	**Range**	
Independent variables (factors)	Low (-1)	High (+1)
X1 = Tf -PLGA conc: curcumin (100 mg) (%)	5	20
X2 = Stirring speed (rpm)	1	10
X3 = Emulsifying conc. (%) (%)	5	10

Table 2: Formulation and Optimization of Microemulsion system (MES)

Run	**Tf-PLGA Conc.: Curcumin (100mg) (%)**	**Stirring speed (rpm)**	**Emulsifying conc. (%)**	**Mean particle size (nm)**	**Entrapment efficiency**
1	3	500	1	402.5	85.4
2	5	1250	1	238.5	97.5
3	**5**	**1250**	**10**	**214.8**	**99.7**
4	1	500	5.5	402.8	74.8
5	3	1250	5.5	298.7	89.5
6	3	500	10	395.4	84.7
7	3	1250	5.5	269.8	95.2
8	1	1250	10	203.7	79.5
9	3	1250	5.5	228.7	88.7
10	**5**	**2000**	**5.5**	**248.9**	**98.7**
11	3	1250	5.5	220.8	85.8
12	3	1250	5.5	225.3	89.5
13	3	2000	1	318.9	84.9
14	1	2000	5.5	238.8	81.2
15	**5**	**500**	**5.5**	**325.7**	**99.2**
16	1	1250	1	285.2	82.5

17	3	2000	10	228.7	91.7

RESULT AND DISCUSSION

Statistical Significance on Particle size

Effect of Independent variable on mean particle size can be demonstrated in **table no. 3** in which the response model is following the quadratic model. Quadratic model helps identify the optimal combination of ingredients and their levels to achieve the desired particle size properties. This is critical for ensuring that a formulation meets the required specifications. The R^2 value in form of adjusted and predicted value having the difference is 0.2 ensuring the research follows the quadratic model[10-11].

Table N3: Mean particle size of nanoparticle

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	R^2	Adeq Precision	Std. Dev.	Mean	C.V. %	
Linear	0.0263	0.1450	0.3803	0.0684						
2FI	0.7191	0.1007	0.2913	-0.7673						
Quadratic	**0.0027**	**0.9875**	**0.9907**	**0.9701**	0.9947	**9.6304**	**26.22**	**279.25**	**9.39**	**Suggested**
Cubic	0.9875		0.7465							Aliased

Table no.3 depicts that the **Predicted R^2** of 0.9701 is in reasonable agreement with the **Adjusted R^2** of 0.9907; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 9.630 indicates an adequate signal. The standard deviation was observed 26.22 and average mean particle size found 279.25. also suggested the equation followed the quadratic model which is best fit model for this study. This model can be used to navigate the design space. Select the highest order polynomial where the additional terms are significant and the model is not aliased. Once the best fit model quadratic was found suitable for this study further statistical analysis were started.

ANOVA for Quadratic model

Table N4: ANOVA for Quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	68882.32	9	7653.59	**11.13**	0.0022	significant
A-Tf-PLGA Ratio	1315.85	1	1315.85	1.91	0.2091	
B-Stirring speed	30147.40	1	30147.40	43.84	0.0003	
C-Emulsifying agent conc.	5125.78	1	5125.78	7.45	0.0293	
AB	1900.96	1	1900.96	2.76	0.1403	
AC	835.21	1	835.21	1.21	0.3069	
BC	1726.40	1	1726.40	2.51	0.1571	
A^2	2172.99	1	2172.99	3.16	0.1187	
B^2	25687.50	1	25687.50	37.36	0.0005	
C^2	388.65	1	388.65	0.5652	0.4767	
Residual	4813.50	7	687.64			
Lack of Fit	142.33	3	47.44	0.0406	0.9875	not significant
Pure Error	4671.17	4	1167.79			
Cor Total	73695.82	16				

Table No.4 depicts the **Model F-value** of 11.13 implies the model is significant. There is only a 0.22% 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, B^2 are significant model terms. As the p- value of B, C, B^2 are 0.0003, 0.0293 and 0.0005 respectively which is less than 0.0500 indicates that emulsifying agent concentration and stirring speed induce the valuable impact on particle size than other concentration. In the case of residual the p-value more than 0.05 i.e 0.9875, gives the indication of no significance of the model. The **Lack of Fit F-value** of 0.04 implies the Lack of Fit is not significant relative to the pure error. There is a 98.75% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good, that want the model to fit[12-13].

Coefficients in Terms of Coded Factors

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 14.000 and 13.000; VIFs greater than 10, it suggests a strong correlation with other independent variables in the model.

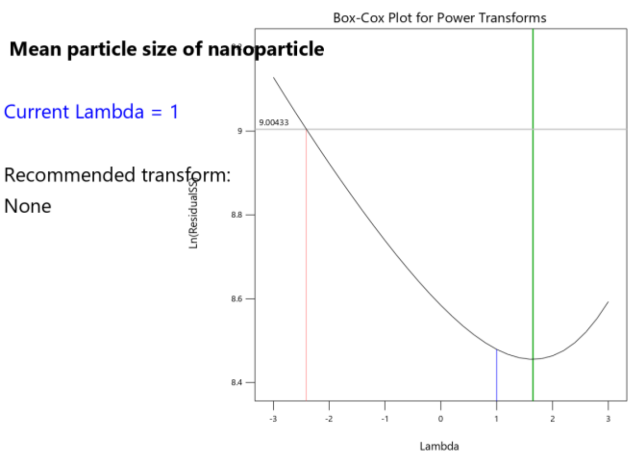
While VIF are 1.00, indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable. So, the stirring speed and emulsifying agent concentration have strong corelation with drug particle size and it vary the particle size in case of changing the value of independent variable. That is also shown in Table No.5

Table 5: Coefficients in Terms of Coded Factors

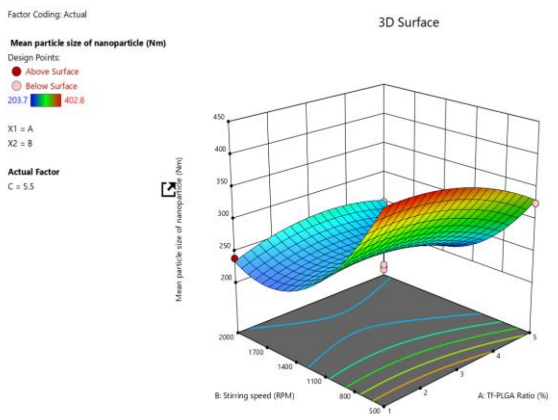
Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	248.66	1	11.73	220.93	276.39	
A-Tf-PLGA Curcumin Ratio	+12.82	1	9.27	-34.75	9.10	1.0000
B-Stirring speed	-61.39	1	9.27	-83.31	-39.46	13.0000
C-Emulsifying agent conc.	-25.31	1	9.27	-47.24	-3.39	14.0000
AB	21.80	1	13.11	-9.20	52.80	1.0000
AC	14.45	1	13.11	-16.55	45.45	1.0000
BC	-20.77	1	13.11	-51.78	10.23	1.0000
A^2	-22.72	1	12.78	-52.94	7.50	1.01
B^2	78.11	1	12.78	47.89	108.33	1.01
C^2	9.61	1	12.78	-20.61	39.83	1.01

Final Equation in Terms of Coded Factors = +248.66--12.82A+21.39B+14.31C-21.80 AB-14.45 AC+20.77 BC-22.72A^2+78.11 B^2+9.61 C^2……. (Eq.)

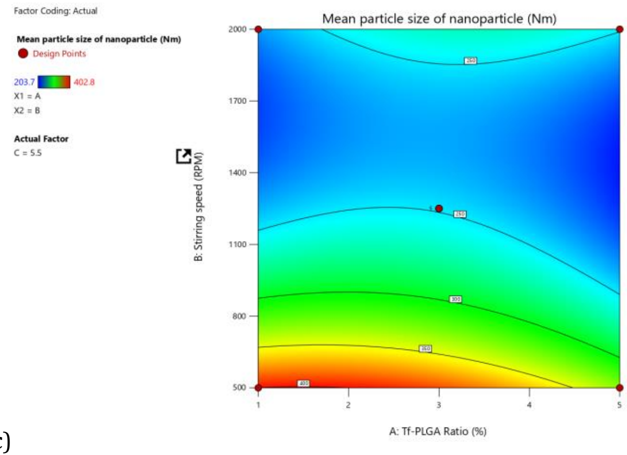
The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The Equation no.1 depicts that individual factor like B (Stirring speed) and C (emulsifying agent conc.) gives the positive impact on the particle size with the value of +21.39B ,14.31C respectively. However, the impact was analysed combined with Tf-PLGA Curcumin ratio, stirring speed, and emulsifying agent concentration, and the value obtained -21.80AB and -14AC, shown the negative impact on particle size. The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the centre of the design space.



(a)



(b)



(c)

Figure 1: (a) Box-cox plot for power transformation (b) 3D surface graph for mean particle size of nanoparticles (C) Contour graph for mean particle size.

Figure No.1 Shows the all three types of graphs and they show the mean particle size of nanoparticles. The graphs have shown that impact of all three independent variables given the impact of mean particle size. As shown that stirring speed given the positive impact on particle size. The optimum speed (1200 rpm) as shown in Table no.2 influence the optimum size of particles which is also shown in Figure no.1

Statistical Significance on Entrapment Efficiency (EE)

Impact of independent variables like Tf-PLGA Curcumin Ratio, stirring speed and emulsifying agent concentration over the impact of EE. All statistical tools were employed to found out the all-independent variables which are shown in Table. No 5[14-15].

Table 5: Entrapment efficiency of nanoparticle

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	R^2	Adeq Precision	Std. Dev.	Mean	C.V. %	
Linear	**< 0.0001**	**0.6961**	**0.9910**	**0.9892**	0.9925	**14.9890**	**3.04**	**88.71**	**3.43**	**Suggested**
2FI	0.3479	0.7307	0.8395	0.7218						
Quadratic	0.4817	0.7162	0.8352	0.6137						
Cubic	0.7162		0.7874							**Aliased**

From the Table No.5, the **Predicted R^2** of 0.9892 is in reasonable agreement with the **Adjusted R^2** of 0.9910; i.e. the difference is less than 0.2 which support the significance of the study and impact of the independent variable over dependent variables. **Adeq Precision** measures the signal to noise ratio. A ratio greater than 4 is desirable but for this study the ratio was calculated as14.989 indicates an adequate signal. This model can be used to navigate the design space. Select the highest order polynomial where the additional terms are significant and the model is not aliased. Therefore, linear model was fit for study as shown in table n.5. A linear model is a type of mathematical model used in statistics and mathematics to describe the relationship between a dependent variable and one or more independent variables. In a linear model, it is assumed that this relationship is linear, meaning that a change in the independent variable(s) is associated with a constant change in the dependent variable.

ANOVA for Linear model

Table 6: Entrapment efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	755.08	3	251.69	27.18	< 0.0001	significant
A-Tf-PLGA Ratio	735.36	1	735.36	79.41	< 0.0001	
B-Stirring speed	17.41	1	17.41	1.88	0.1936	
C-Emulsifying agent conc.	2.31	1	2.31	0.2496	0.6257	
Residual	120.38	13	9.26			
Lack of Fit	73.85	9	8.21	0.7054	0.6961	not significant

Pure Error	46.53	4	11.63
Cor Total	875.46	16	

Table No.6 depicts the **Model F-value** of 27.18 implies the model is significant. There is only a 0.22% 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 A, is significant model terms. As the p- value of A is <0.0001 which is less than 0.0500 indicates that Tf-PLGA Ratio induce the valuable impact on entrapment efficiency. In the case of residual the p-value more than 0.05 i.e 0.6961, gives the indication of no significance of the model. The **Lack of Fit F-value** of 0.7054 implies the Lack of Fit is not significant relative to the pure error. The **Lack of Fit F-value** of 0.71 implies the Lack of Fit is not significant relative to the pure error. There is a 69.61% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit[16].

Coefficients in Terms of Coded Factors

Table 7: Coefficients in Terms of Coded Factors

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	88.71	1	0.7380	87.12	90.31	
A-Tf-PLGA Curcumin Ratio	9.59	1	1.08	7.26	11.91	12.0000
B-Stirring speed	1.48	1	1.08	-0.8493	3.80	1.0000
C-Emulsifying agent conc.	0.5375	1	1.08	-1.79	2.86	1.0000

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 12.000; VIFs greater than 10, it suggests a strong correlation with other independent variables in the model While VIF are 1.00, indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable. So. The Tf-PLGA Ratio has strong corelation with drug entrapment efficiency and while concentration increased the entrapment efficiency will also be increased that is also shown in Table No.5[17].

Final Equation in Terms of Actual Factors =
+71.21524+4.79375 Tf-PLGA- Curcumin Ratio-0.001967 Stirring speed-0.119444 Emulsifying agent conc………. (Eq.2)

The equation in terms of actual factors was used to make predictions about the response for given levels of each factor. Here, the levels specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space. The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the VIFs are 1; VIFs greater than 1 indicate multi-colinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable. From the equation (2) the Tf-PLGA- Curcumin Ratio has the positive impact on EE with given value (4.79375) while other two factors, stirring speed (-0.001967) and emulsifying agent concentration (-0.119444) have given the negative values which indicates the slightly negative impact on EE. So, Tf-PLGA-Curcumin Ratio has given the positive impact on EE.

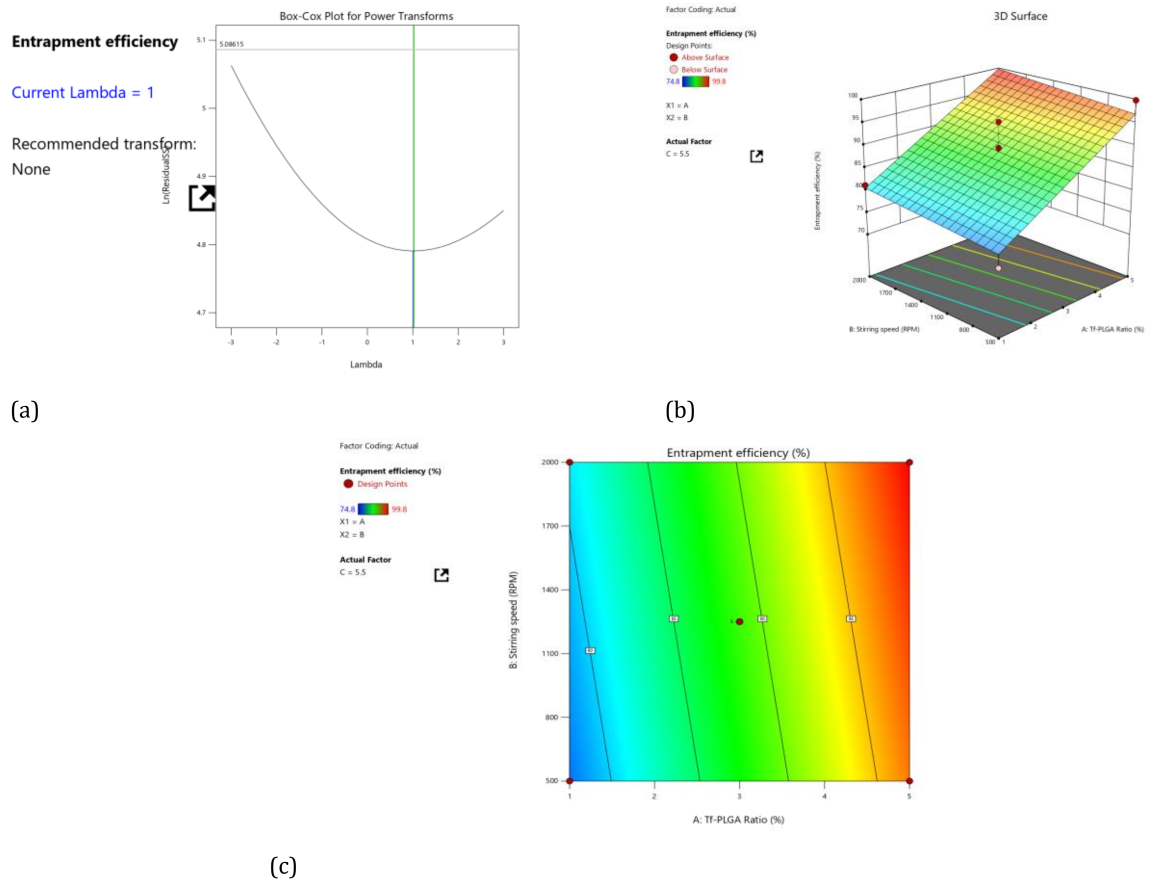


Figure 2: (a) Box-cox plot for power transformation (b) 3D surface graph EE nanoparticles (C) Contour graph for EE.

Figure No.2 Shows the all three types of graphs and they show the EE of nanoparticles. The graphs have shown that impact of all three independent variables given the impact of EE. As shown that Tf-PLGA Curcumin Ratio given the positive impact on EE. The optimum Concentration (5 mg) as shown in Table no.2 influence the optimum EE, which is also shown in Figure no.2

SUMMARY AND CONCLUSION

The topic Screening the statistical impact of some independent variables on the particle size and entrapment efficiency (EE) for the design and development of curcumin-loaded PLGA-Tf nanoparticles is a statistical approach for designing the formulation using the Box-Behnken 3^2 model. The research was emphasised to find out the three independent variables, like Tf-PLGA Curcumin ratio, and dependent variables like particle size and EE of the drug. Design Expert Version 13.1.5 was used to analyse the impact of independent variables over dependent variables. The statistical data were interpreted to provide insights, and it was observed that stirring speed has an impact on particle size. When the stirring speed is increased, the nanoparticle size decreases gradually, whereas the Tf-PLGA-curcumin ratio has an effect on EE. In this situation, as the concentration of Tf-PLGA-curcumin increases to a certain level, EE has also increased, i.e., the more concentration of drug will be enclosed in the coating material. This statistical exercise will have a lot of impact on formulation development prospects because it will save researchers money and time in developing nanoparticles.

AKNOWLEDGEMENT

The author very grateful to Varav biogenesis Ltd. Kala-Amb, H.P. India for providing financial support and gifted some valuable chemical for this project. The author also thankful to Himalayan Institute of Pharmacy for providing their lab and instrument facility.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest related to the research presented in this paper titled " Screening the statistical impact of some independent variables on the particle size and entrapment efficiency (EE) for the design and development of curcumin-loaded PLGA-Tf nanoparticles.

REFERENCES

1. Sharma A, Babu Sharma RB, Verma A, Thakur R. Insight on nanoparticles, green synthesis and applications in drug delivery system: A comprehensive review. Int J Life Sci Pharm Res. 2022; 12(5):68-84. https://doi.org/10.22376/ijpbs/lpr.2022.12.5.P68-84

2. Gupta D, Sharma R, Goel S. Synergistic effect of antibacterial activity by amalgamation of silver nanoparticles with cephalosporin against antibiotic resistant. Int J Pharm Res. 2022 Oct1; 14(4):(09752366).

3. Elisetti Sk, Arora V, Sharma Rb. Polymers for designing 3D Printed Pharmaceutical Products. J Res Pharm. 2023 Mar 1; 27(2).

4. Sharma RB, Arora V, Arora S, Kapila A, Sharma R. The effect of biomaterials of cross carmellose sodium and Eudragit S-100 as model polymers on colon targeted drug release in different dosage forms. Mater Today Proc. 2022 Jan 1; 48:1638-44. https://doi.org/10.1016/j.matpr.2021.09.526

5. Esfandiarpour-Boroujeni S, Bagheri-Khoulenjani S, Mirzadeh H, Amanpour S. Fabrication and study of curcumin loaded nanoparticles based on folate-chitosan for breast cancer therapy application. Carbohydr Polym. 2017 Jul 15; 168:14-21. https://doi.org/10.1016/j.carbpol.2017.03.031 PMid:28457434

6. Sharma M, Dhiman N, Singh P, Sharma R, Sharma RB, Arora V et al. Gel incorporated lipid nanoparticles for the treatment of psoriasis. Mater Today Proc. 2022 Jan 1; 48:1690-701. https://doi.org/10.1016/j.matpr.2021.10.023

7. Sharma R, Ranawat MS, Sayra B, Bhandar A, Chouhan CS. Statistical screening of starch paste and guar gum on hardness and

disintegration time of fast dissolving tablet. Int J Pharm Pharm Sci. 2012; 4:4.

8. Sharma R, Choudhary D, Bhandari A, Verma R, Ranawat MS. A comparative study of novel super disintegrating agent, guar gum to existing super disintegrating agent, sodium starch glycolate on release rate of drug from fast dissolving tablet. J Pure Appl Sci Technol. 2013 Jul 1; 3(2).

9. Katageri SB, Sharma R. Development and Optimization of Self-Nanoemulsifying tablet dosage form of nateglinide using Box-Behnken design. J Pharm Sci. 2016. 10. Monnier O, Klein JP, Ratsimba B, Hoff C. Particle size determination by laser reflection: methodology and problems. Part Part Syst Charact. 1996 Feb; 13(1):10-7. https://doi.org/10.1002/ppsc.19960130104

11. Vashist H, Sharma RB, Sharma D, Upmanyu N. Pharmacological activities on Zanthoxylum armatum-A review. World J Pharm Pharm Sci. 2016; 5(12):408-23.

12. Wadhwa R, Usman MR, Sharma RB, Tomar S. Exploring cocrystals of imatinib: synthesis, characterization, and in vitro evaluation. J Surv Fish Sci. 2023 Jul 12:734-43.

13. Sharma RB, Kashyap D, Thakur H. Updated review on proniosomal transdermal drug delivery system. Int J Drug Dev Res J. 2023; 15(1):989.

14. Li X, Wang L, Wang B. Optimization of encapsulation efficiency and average particle size of Hohenbuehelia serotina polysaccharides nanoemulsions using response surface methodology. Food Chem. 2017 Aug 15; 229:479-86. https://doi.org/10.1016/j.foodchem.2017.02.051 PMid:28372204

15. Thakur D, Sharma R. Solid dispersion a novel approach for enhancement of solubility and dissolution rate: a review. Indian J Pharm Biol Res. 2019 Sep 30; 7(3):5-11. https://doi.org/10.30750/ijpbr.7.3.2

16. Singh J, Sharma RB, Mehan N, Beniwal SK. Itraconazole-loaded nanocrystals development and characterization for the treatment of ophthalmic fungal infection. Lat Am J Pharm. 2023 Jul 19; 42(3):839-47.

17. Dahiya A, Sharma RB. Biomaterial role and application in polymer science. AIP Conf Proc. 2023 Feb https://doi.org/10.1063/5.0130466