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3^2 Full Factorial Design for Optimization of Clindamycin Phosphate Loaded Nanogel: A Design of Experiments (DOE) Approach

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ABSTRACT

Clindamycin is the First choice of antibiotic for the treatment of streptococcal gangrene. Streptococcal Gangrene is a rare infection that means "decaying infection of the fascia," which is the soft tissue that runs throughout the body. Clindamycin belongs to BCS class III that is high solubility, and low permeability with a protein binding of 95%, and a topical bioavailability of 4-5%. At present Eudragit L 100 was used as a release retardant polymer and Propylene glycol as a penetration enhancer. Nanogels were prepared by Nanoprecipitation and Dispersion method by changing the drug-polymer ratio (1:0.05, 1:0.1, 1:0.15) and process parameters were optimized. Optimization studies for the given procedure were performed by using 3^2 full factorial designs (Design Expert Software Trial version10.0.7), and 13 runs were generated. The concentration of Eudragit L100 (X1) and Carbopol 934(X2) was selected as independent variables. Particle size (R1), PDI (R2), and % Drug release (R3) were selected as dependent variables. Application of such optimizing technique[5]provides an efficient and economical method to gain the essential information and thus to understand the relationship between controllable independent variables and dependent variables or responses in terms of performance and quality of Nanogels produced. The drug-loaded nanogels were evaluated for drug release, PDI, and particle size. The best-standardized formulation F42 was obtained. Thus, it was concluded that Clindamycin can be formulated as Nanogels that can release the drug for up to 24hrs, promising drug delivery for topical application as being more useful than conventional formulation therapy.

1. INTRODUCTION

Streptococcal Gangrene is a rare infection that means "decaying infection of the fascia". Streptococcal gangrene also termed streptococcal necrotizing fasciitis, is resurgent but remains exceedingly rare[1,2,3]. It is a rare disease but dangerous as the spread of infection affects the major blood vessels, which can cause sepsis, systemic toxic reaction, and compromise the functioning of the vital organs. Drugs of choice used in treating necrotizing fasciitis are Clindamycin(26.8%), Vancomycin(25.5%), Meropenem(6.1%), Cilastatin(6.1%), Piperacillin(5.6%), Daptomycin(4.8%), Metronidazole(2.6%), Ampicillin(2.2%), Others(16.9%). Out of all, Clindamycin is the first choice of antibiotic for the treatment of Nacrotizing Fascitis. Clindamycin is used primarily to treat anaerobic infections caused by susceptible anaerobic bacteria, including dental infections, infections of the respiratory tract, skin, and soft tissue, and peritonitis. As Clindamycin is the first choice of drug for the treatment of streptococcal gangrene and it has low topical bioavailability of 4-5% hence, it became an ideal candidate for the study. Effective targeted drug delivery systems have been a dream for a long time, but it has been largely frustrated by the complex chemistry that is involved in the development of new systems. Nanogels are robust nanoparticles that could be used to deliver active drug compounds in controlled drug delivery applications. Nanogels drug delivery system is more effective and safer for both hydrophilic and hydrophobic drugs due to their chemical composition and formulations that are inappropriate for other formulations. Nanogels have enabled the enlargement of functionalized nanoparticles, which act as drug carriers that can be loaded with drugs and other active material to be released in a controlled manner at a specific site. The nanogels-based formulations are said to have better drug release and the nanogels system has better penetration of drug hence we can speculate that clindamycin phosphate-loaded nanogels formulation is a good candidate for the topical delivery for the treatment of *Streptococcal Gangrene*. The choice and optimization of formulation and process variables are of great importance in the pharmaceutical industry. Design of Experiments (DoE) has arisen as a tool for improved quality risk management, which starts with predefined aims and emphasizes a better understanding of product and process parameters. 3^2Full factorial design is a simple systematic design style in the design of Experiments that allows us to understand the estimation of main effects and interactions. The use of Optimization techniques enhances the predictability of the dosage form, a response surface methodology (RSM) is a widely acknowledged DoE tool to extract maximum information from well-designed experiments.

2 MATERIAL AND METHODS

2.1 Chemical and Reagents

Clindamycin Phosphate was a gift sample from Mylan laboratory Ltd. Eudragit L 100, Polyvinyl alcohol, Dichloromethane, Triethanolamine, Di-sodium hydrogen orthophosphate, Glycerine Propylene glycol, Potassium dihydrogen orthophosphate were procured from SD Fine Chemicals Ltd., Carbopol, Methanol from Himedia Laboratory Pvt. Ltd, Mumbai, Sodium hydroxide pellets from Qualigens fine chemicals, Disodium Hydrogen Phosphate Leo chem S.D fine chemicals limited, Mumbai.

2.2 Preparation of clindamycin Nanogel

Clindamycin Phosphate nanogels were prepared by Nanoprecipitation and the dispersion method was Eudragit L100 and Tween 80 as a stabilizer. The drug and polymer were taken and mixed in glycerol with continuous stirring on a magnetic stirrer which showed more stability and no flocculation or sedimentation was observed. The drug and polymer were completely dissolved and nanoparticles were prepared by the Nanoprecipitation method. The gel was prepared by Dispersion method using Carbopol 934 it was kept in water for hours for swelling. The Carbopol was swelled and kept on a magnetic stirrer for stirring. The drug equivalent amount or the separated nanoparticulate system was added to the Carbopol mixture along with Propylene glycol which acts as a penetration enhancer, triethanolamine was added to the nanogel mixture to maintain the pH of the formulation and finally, the nanogels were formed.

2.3 Optimization using Design Expert Software

Optimization studies for the given procedure were performed by using 3^2 full factorial designs (Design Expert Software Trial version10.0.7), and 9 runs were generated. The concentration of Eudragit L100 (X1) and Carbopol 934(X2) was selected as independent variables. Particle size (R1), PDI (R2), and % Drug release (R3) were selected as dependent variables. The formulations were coded as F31, F32, and F33&F39, and 4 control formulations were given by the model. Three different concentrations of the polymer i.e. 5, 10, and 15 mg were selected for the optimization studies. It signifies how the responses change when the two factors are changed concurrently. The experiment was carried out at three different levels -1, 0, and +1 wherein -1, 0, and +1 depict low medium, and high concentrations respectively.

Table: 1 Coded values for Independent variables

Variables	F31	F32	F33	F34	F35	F36	F37	F38	F39	F40	F41	F42	F43
X1	-1	-1	0	1	0	1	-1	1	-1	0	1	1	-1
X2	0	-1	0	1	-1	0	-1	-1	1	1	1	-1	1

Table: 2 Coded values and actual values for Independent variables

Coded Values	Actual Values	
	XI (mg)	X2 (mg)
-1	5	0.05
0	10	0.1
1	15	0.15

2.4 Data optimization

a) Generation of a polynomial equation- Various response surface methodology (RSM) computations for the current optimization study were performed employing Design Expert software. Polynomial models including interaction were generated for all the response variables using the multiple linear regression analysis (MLRA) approaches.

b) Statistical Analysis of Data- The effect of independent variables on the responses was checked by using a statistical tool that is ANOVA using Design Expert software (version 10.0.7). A value of $p < 0.05$ was considered statistically significant.

c) Generation of 3D response surface plots-For the measured responses, three-dimensional plots were generated to determine the change of the response surface. These types of plots are beneficial in the study of the effect of two factors on the response at one time. It signifies the effect of independent variables on the responses by the graphical presentation.

3 RESULTS AND DISCUSSION

Table: 3 Formulation Parameters for Optimization of the Nanogel

Formulation Code	Run	Factor 1 Eudragit L100	Factor 2 Carbopol 934(%)	Response 1 Particle size (nm)	Response2 P.D.I	Response 3DrugRelease(%)
F31	1	5	0.05	580	0.481	54.4
F32	2	5	0.1	530.2	0.504	59
F33	3	5	0.15	498	0.627	65
F34	4	15	0.1	438	0.435	77.4
F35	5	10	0.1	428	0.498	64
F36	6	15	0.15	421	0.383	80.12
F37	7	15	0.05	480	0.745	54.1
F38	8	10	0.5	461	0.657	42
F39	9	10	0.15	435	0.601	72
F40	**10**	**15**	**0.133**	**420**	**0.381**	**81.04**
F41	**11**	**14.94**	**0.135**	**421**	**0.380**	**83.30**
F42	**12**	**14.97**	**0.137**	**419**	**0.379**	**84.21**
F43	**13**	**14.95**	**0.143**	**423**	**0.384**	**82.03**

3.1 Generation of Polynomial equations

The statistical model generated interactive polynomial terms for each response, equations are as follows: -

$$Y = \beta0 + \beta1\ A + \beta2\ B + \beta3\ AB + \beta4\ A2 + \beta5\ B2$$

Where, Y is the independent variable, β0 is the arithmetic mean response of the 9 runs and β1 is the estimated co-efficient for the factor A the main effects of the amount of A and B signify the average result when the factors were changed one at a time from their lower to higher values. The interaction terms (AB) show how the response changes when two factors are concurrently changed. The data obtained from DOE strongly signifies that particle size, PDI and %DR is dependent on the selected independent variables. Conclusions can be drawn

from the following polynomial equations depending on the mathematical sign it carries that is a positive and negative sign, indicating synergistic and antagonistic effects.

- Y1 – (PS) = 430.89 –47.17 A –30.17 B + 2.25 AB + 49.17 A2 + 13.17B2
- Y2– (PDI) = 0.52–0.018A –0.056B –0.14AB –0.066 A2 +0.094 B2
- Y3 – (%DR) = 62.57+ 6.21A + 11.81B –4.91AB + 6.34 A2 + 4.86 B2

Where, A= Concentration of Eudragit L100

B= Concentration of Carbopol 934

The main effects of the amount of A and B signify the average result when the factors were changed one at a time from their lower to higher values. The interaction terms (AB) show how the response changes when two factors are concurrently changed. The polynomial equation claimed that as the concentration of polymer and the gelling agent is increased then the particle size of the formulation is decreased and has less PDI than 0.5 nm and the % drug release is increased.

3.2 Statistical analysis of data

Analysis of variance (ANOVA) was applied to recognize insignificant factors. Data were evaluated using Design-Expert Software (version 11.0). From the data obtained it was evident that the p-value was found to be less than 0.05 ($p<0.05$) for all the dependent variables. Model F value for PS, PDI, and %DR was found to be 14.01, 16.67, and 12.47 respectively which implies the model is significant. R- Squared is a goodness-of-fit measure for linear regression models. This statistic indicates the percentage of the variance in the dependent variable that the independent variables explain collectively.

Table-4 ANOVA for quadratic models model-F value of Particle Size(Y1)

Source	Sum of squares	df	Mean Square	value	p-value Prob >F	
Model	24010.03	5	4802.01	14.01	0.0272	**Significant**
A-EUDRAGITL100	13348.17	1	13348.17	38.95	0.0083	
B-CARBOPOL934	5460.17	1	5460.17	15.93	0.0282	
AB	20.25	1	20.25	0.059	0.8236	
A^2	4834.72	1	4834.72	14.11	0.0330	
B^2	346.72	1	346.72	1.01	0.3886	
Residual	1028.19	3	342.73			
Cor Total	25038.22	8				

Table 5: ANOVA for quadratic models indicating-Squared value Model-F value of Particle Size

Std. Dev.	18.51		**R-Squared**	0.9589
Mean	472.44		**Adj R-Squared**	0.8905
C.V.%	3.92		**Pred R-Squared**	0.5259
	11869.57		**Adeq Precision**	10.513

Table 6- ANOVA for quadratic models of PDI(Y2).

Source	Sum of squares	df	Mean Square	F Value	p-value Prob>F	
Model	0.13	5	0.026	16.67	0.0213	**Significant**
A-EUDRAGITL100	2.053E-003	1	2.053E-003	1.34	0.3314	
B-CARBOPOL 934	0.019	1	0.019	12.10	0.0401	
AB	0.081	1	0.081	52.84	0.0054	
A^2	8.668E-003	1	8.668E-003	5.64	0.0981	
B^2	0.018	1	0.018	11.42	0.0431	
Residual	4.611E-003	3	1.537E-003			

Table 7- ANOVA for quadratic models indicating R-Squared value Model-F value of PDI.

Std Dev.	0.039		**R-Squared**	0.9652
Mean	0.54		**Adj R-Squared**	0.9073
C.V.%	7.25		**Pred R-Squared**	0.6317
	0.049		**Adeq Precision**	12.381

Table 8-ANNOVAforquadratic models model-F value of%Drug Release (Y3).

Source	**Sum of squares**	**df**	**Mean Square**	**F Value**	**p-value Prob>F**	
Model	1291.12	5	258.22	12.47	0.0320	**Significant**
A-EUDRAGITL100	231.01	1	231.01	11.16	0.0444	
B-CARBOPOL934	836.15	1	836.15	40.39	0.0079	
AB	96.33	1	96.33	4.65	0.1199	
A^2	80.35	1	80.35	3.88	0.1434	
B^2	47.27	1	47.27	2.28	0.2279	
Residual	62.11	3	20.70			
Cor Total	1353.22	8				

Table 9- ANOVA for quadratic models indicating R-Squared value Model-F value of % drug Release.

Std. Dev.	4.55	**R-Squared**	0.9541
Mean	63.56	**Adj R-Squared**	0.8776
C.V.%	7.16	**Pred R-Squared**	0.4565
	735.43	**Adeq Precision**	11.053

3.3 Generation of 3D Response Surface Plots.

For the measured responses, three-dimensional plots were generated to determine the change in the response surface. These plots generated were found to be beneficial in the study of the effect of two factors on the response at one time. Surface plots generated were in agreement with the polynomialtermgeneratedindicatingtheeffectofconcentrationofEudragitL100(X1) and

concentration of carbopol 934 (X2) on responses. 3D plots are depicted in Figures 1 to 5indicating the effect of independent variables on the response of dependent variables. From the 3D response plots generated for particle size (Y1) depicted in figure 2, the graph represents that there is decrease in particle size with increase in the concentration of eudragit L 100 and decreasing in the concentration of carbopol 934. The counter plots generated for particle size as depicted in figure 1, also indicates the same decrease in particle size is observed with increasing the concentration of polymer and gelling agent.

From the 3D response plots generated for P.D.I (Y2) depicted in Figure 3, the graph represents that there is a decrease in PDI with considerable increase in concentration of polymer and gelling agent.

From the 3D response plots generated for (Y3) % drug release (%DR), depicted in figures 4 & 5, the graph represents that there was an increase in an initial increase in the concentration of eudragit L 100 and carbopol 934 there was a considerable increase in % drug release possibly due to combination of the same concentration of polymer and gelling agent due to high concentration of polymer. Eudragit L 100 showed a variable effect on %drug release. Initially decrease in the concentration of eudragit l 100 in formulation showed a decrease in % drug release but with the same concentration again it showed a higher % drug release possibly because the combined concentration of polymer and the gelling agent has a higher effect on % drug release.

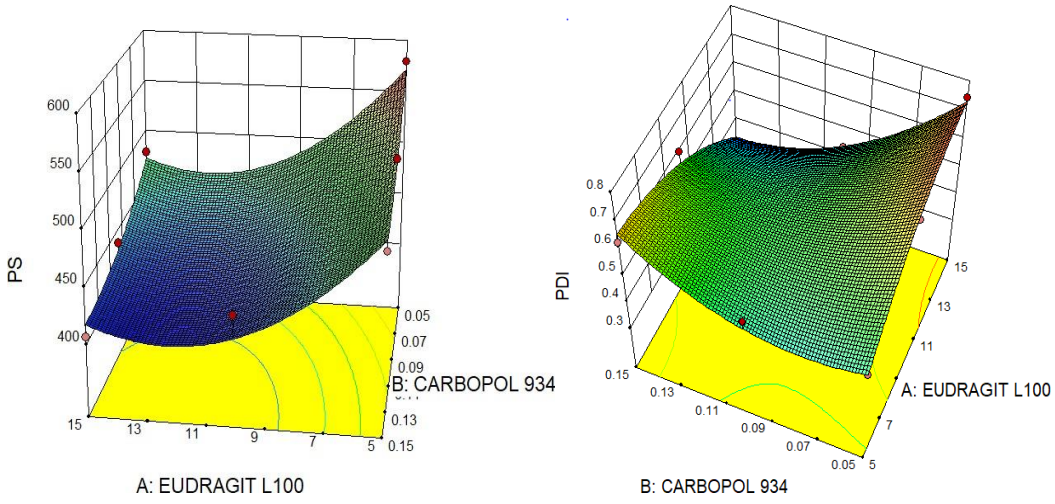

Figure 1. Counter plot exhibiting the effect to Eudragit L100 and Carbopol 934 Concentration on Particle Size (nm) &PDI

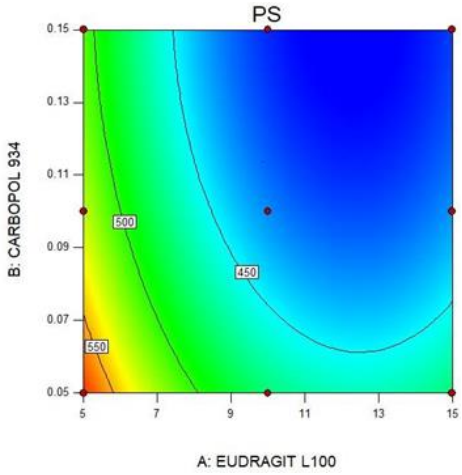

Figure 2. 3D Response Surface Plots for particle size & PDI.

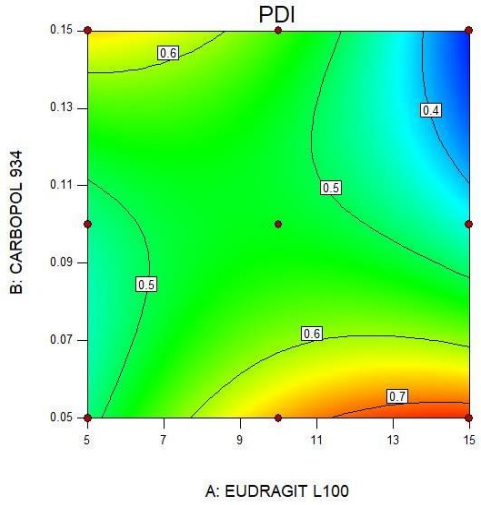

Fig 3: Surface plot exhibiting the effect of Eudragit L100 and Carbopol 934 Concentration on PDI

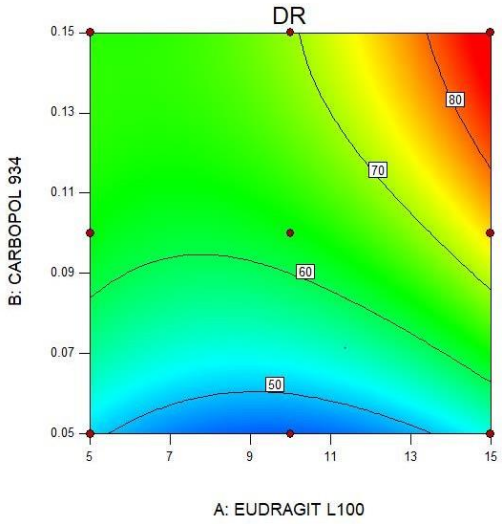

Fig 4: 3D Response Surface Plots of Eudragit L100 and Carbopol 934 for % Drug Release

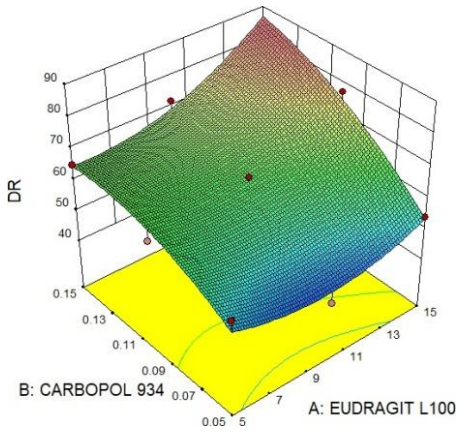

Fig 5: Contour Plots for % DR Signifying the effects of two factors on response.

Based on an acceptable Particle size range which showed the highest drug release obtained from 3^2 (2 factors and 3 levels) full factorial design, formulation 42(F42) which had a good PDI range was selected as an optimized formulation and further carried out for evaluation studies.

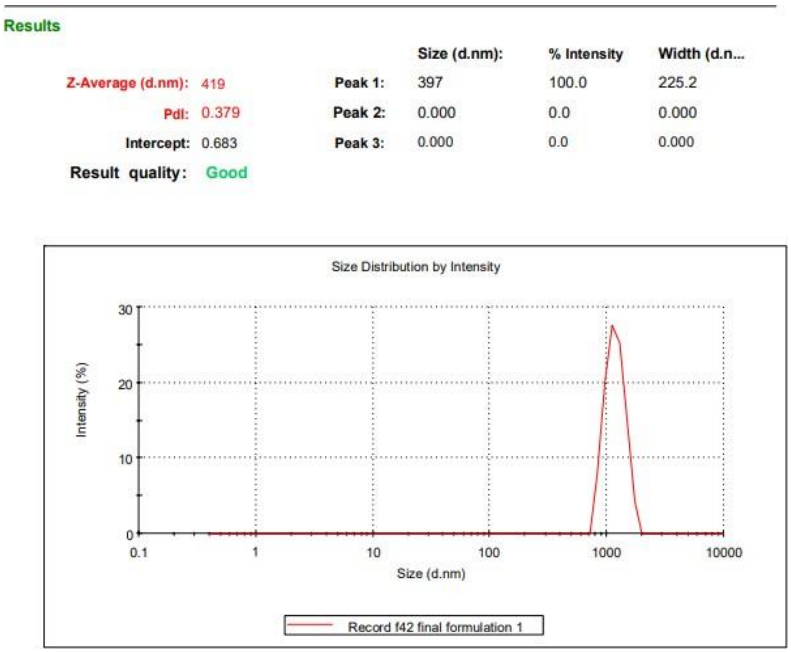

Figure 6-Particle Size PDI of Optimized Formulation

4 CONCLUSION:

Design Expert software was used to apply full factorial design to study the response surface of a 3-level factorial design with 13 runs in a quadratic model. The formulations were fabricated according to a3^2 full factorial design, allowing the simultaneous evaluation of two formulation variables and their interaction. Based on the preliminary studies, the two

independent variables included were gelling agent concentration and polymer concentration was taken. The two variables were compared over 3levels, +1(High), 0(Medium), and -1(Low). The effect of the two factors on the response of the dependent variable i.e, Y1(Particle Size), Y2(P.D.I), and Y3(% Drug release) was studied by a polynomial equation. The use of 3^2 full factorial design showed significant findings which helped in the successful development of Clindamycin Nanogel for topical delivery. Comparing the actual and predicted responses indicated that the surface response methodology is suitable to make optimization of Clindamycin Nanogel to produce a good dosage form in terms of Particle size and Drug Release. Optimization is an advanced experimental process, like those used in nanoparticle development, leading to robust preparation techniques with a reproducible product having desired properties. Further, factorial designs are the most commonly hired method to optimize experiments and to identify which factors dominate the output and what level of these variables guide for a better and desired Dosage Form.

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