Fabrication and Optimization of Lincosamide antibiotic loaded hydrogel: A Design of Experiments (DOE) Approach using Minitab software

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Abstract:

Utilising Minitab software, the current work attempted to maximise the circumstances for the creation and production of a Nanosponge-loaded hydrogel for a model Lincosamide antibiotic. Methodology: Factorial design was used to optimise the drug:polymer ratio and surfactant concentration. A two component, three level full factorial design was created and carried out in a fully randomised sequence to examine all potential combinations of both factors at all levels. By varying the drug polymer ratio (1:0.05, 1:0.1, and 1:0.15), nanosponges were made using the emulsion solvent diffusion method, and process variables were optimised using a 32 complete factorial central design. The Minitab® database used design of experiment (DOE), which predicted the optimal parameters by assessing the combined influence of various elements at once, to achieve the ideal particle size, drug release, and entrapment efficiency. The most effective recipe was chosen to create topical hydrogels containing Carbopol 934 nanosponges.Result and Analysis: The 32 complete factorial design used to create the formulations allowed for the simultaneous study of two formulation variables and their interactions. Surfactant concentration and the ratio of medication to polymer were included as two independent variables based on the early research. The comparison of the two variables was done on three levels: +1 (High), 0 (Medium), and -1 (Low). Polynomial equation was used to investigate the impact of the two factors on the responses of the dependent variables, Y1 (Particle Size), Y2 (Entrapment Efficiency), and Y3 (P.D.I). We assessed the drug-loaded nanosponges' external characteristics, drug content, entrapment effectiveness, and particle size. Response surface methodology (RSM) was utilised in the optimisation phase to examine the significant factors, while the Pareto chart was employed in the screening phase to eliminate the unimportant variables.As a result, it was determined that the Minitab software formulation is a beneficial tool in the creation of Nanosponge hydrogel, which can release the medication for up to 24 hours while having enhanced permeability and tailored release. As a result, medication delivery using prepared nanosponges for topical treatment appears to be more effective than standard formulation therapy.

Keywords: *Optimization, Factorial design, Nanosponges, Hydrogel*

1. Introduction

The infection streptococcal gangrene (SG), often known as flesh-eating disease, causes the soft tissue of the body to die. It is an uncommon bacterial infection of the soft tissue that affects the body's connective tissue network. It is an unadorned illness with an abrupt start and quick spread. Traditionally, an injury to the skin, such as a burn or cut, allows the infection to enter the body. Peripheral vascular disease, diabetes, obesity, cancer, and other conditions that impair immune function are risk factors. (SG) is brought on by an infection of the tissue immediately below the skin (subcutaneous tissue) by a single bacterium (monomicrobial NF) or a large number of germs (polymicrobial NF). When a person contracts the infection, the bacteria or bacterium spreads through the fascia, releasing endotoxins (toxins released as the bacteria die and break down or are lysed) and exotoxins (toxins released by bacteria as waste) that reduce blood flow to tissue (tissue ischemia), enzyme digestion of cells that results in a lesion containing pus and the fluid remnants of dead tissue, and toxins that restrict blood supply to the body's organs. Antibiotics and the body's own defence systems against infection cannot reach these tissues because the blood flow to them decreases.

Surgery is typically required for the therapy, which entails debridement (the surgical removal of infected and dead tissue). The bacteria Staphylococcus aureus frequently cause NF. Streptococcal Gangrene (SG) is mostly caused by a form of these bacteria known as Methicillin-Resistant Staphylococcus Aureus (MRSA). Normally, it does not transmit between people [1,2]. Type 1 infection, Type 2 infection, Type 3 infection, and Type 4 infection are the four subtypes of infection based on the organism that causes the sickness [3,4].

Preferred medications for treating streptococcal gangrene Antibiotics containing lincosamide, such as piperacillin (5.6%), Meropenem (6.1%), Cilastatin (6.1%), and Clindamycin (26.8%). An antibiotic called clindamycin is suggested for use in treating infections brought on by Methicillin-resistant Staphylococcus aureus (MRSA).

A new and evolving technology called a nanosponge allows for regulated medication administration for topical application. Nanosponges are extremely small sponges, around the size of a virus, with an average diameter of less than 1 m.These tiny sponges have the potential to move through the body until they reach the intended target region, where they attach to the surface and start to release the medication in a predictable and controlled manner [5, 6]. They prolong the release of the medication while also increasing its bioavailability. Streptococcal Gangrene can be treated more successfully with nanosponges because they deliver the medication directly to the site of infection rather than circulating throughout the body. However, a number of process variables have an impact on the characteristics of the final nanosponges.

The production of Nanosponges is frequently optimised using the three-level fractional factorial design known as full factorial design. It is based on doing fewer runs of experiments to investigate the impact of various factors on the chosen responses, which saves time and money. Additionally, factorial design is an appropriate technique for examining quadratic response surfaces and constructions based on mathematical models, aiding in the identification of the system's ideal solution.

2. Materials

The lincosamide antibiotic Clindamycin Phosphate was provided free of charge by Mylan Laboratory Ltd. From Mumbai's SD Fine Chemicals Ltd., we ordered ethyl cellulose, dichloromethane, triethanolamine, di-sodium hydrogen orthophosphate, glycerine, propylene glycol, and carbopol. Methanol was provided by Himedia Laboratory Pvt. Ltd. of Mumbai. Hi-Media Ltd. in India supplied the glycerin, propylene glycol, and dialysis membrane; SD Fine Chemicals Ltd. in Mumbai supplied the di-sodium hydrogen phosphate; and Qualigens Fine Chemicals supplied the sodium hydroxide pellets.

3. Methodology

Using the Minitab® programme, a design of experiment (DOE) was conducted in which all variables impacting the three investigated responses were changed simultaneously. The initial variables with the proper levels were chosen using a literature review and preliminary research. Utilising two levels with centre points for numeric variables, full factorial design was utilised to identify the variables that have a significant impact on particle size, drug release, and entrapment effectiveness.

a. Formulation Studies-Preparation of Drug Loaded Nanosponges

Different ratios of ethyl cellulose and polyvinyl alcohol were used in the manufacture of nanosponges utilising the emulsion solvent diffusion method. Following the dissolution of the drug and ethyl cellulose dispersion phase in 20 ml of dichloromethane, 100 ml of an aqueous continuous phase containing polyvinyl alcohol was slowly added. The reaction mixture was first homogenised with Ultra Turrax before being subjected to 20 minutes of sonication with the Probe Sonicator. The created nanosponges were lyophilized and kept in a tightly sealed container [7,8]. The concentration of the retardant material (ethyl cellulose) was varied from 0.05, 0.2 to 0.15%, the surfactant (polyvinyl alcohol) was varied from 0.5 to 1.5 to 2.5, the volume of the external phase (50, 100, and 150 ml), and the internal phase (10, 20, and 30) were standardised while keeping all other parameters constant one at a time in a trial-and-error basis. Stirring speed and sonication time were examined. Homogenization and sonication periods were 30 and 20 minutes throughout the study [9-12]. Table 2 details the formulation study.

Table 1: Standardization of formulation parameters

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Clindamycin Phosphate (gm)	10	10	10	10	10	10	10	101	10	10	10	10
EC (%)	0.05	0.1	0.15	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Internal phase (ml)	20	20	20	20	20	20	20	20	20	10	20	30
External phase (ml)	100	100	100	100	100	100	50	100	150	100	100	100
PVA (%)	2.5	2.5	2.5	0.5	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

Table 2: Effect of formulation parameters on Particle size, Entrapment Efficiency.

Sl. no	Formulation code	Formation of Nano sponges	Particle Size (nm)	Entrapment Efficiency (%)
1	F1	+	72.04	84
2	F2	+	108.4	79
3	F3	+	135.5	73
4	F4	+	384.8	78
5	F5	+	168.3	81
6	F6	+	90.18	83
7	F7	+	296.8	77.87
8	F8	+	216.8	79.02
9	F9	+	385.6	65.4
10	F10	+	249.5	78.4
11	F11	+	186.4	80.56
12	F12	+	378.4	68.98

+ sign indicates formation of nanosponges

b. Optimization using Minitab Software

By utilising a Factorial Design, Mini tab optimised the surfactant concentration and drug polymer ratio. The drug polymer content (X2) and surfactant proportion (X1) were used as independent factors in the study, while the dependent variables were particle size (Y1), entrapment efficiency (Y2), and poly dispersion index (Y3). For the optimisation trials, three alternative surfactant concentrations, 0.5%, 1.5%, and 2.5%, were chosen. Three different combinations of the drug:polymer ratio were used: 1:0.05, 1:0.15, and 1:0.15. Three different levels—-1, 0 and +1—were used for the experiment, where -1, 0 and +1 represent low, medium, and high concentrations, respectively. A total of thirteen formulations, including nine test formulations and four control formulations (Table 3), were created [13-15].

Table 3: Factorial Design Batches for the nanosponge formulations

Variables	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28
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

Table4: Coded values and actual values for Independent variables

Coded values	Actual values	
	X1(%)	X2
-1	0.5	1:0.05
0	1.5	1:0.1
1	2.5	1:0.15

4. RESULTS AND DISCUSSION

Factorial design was used to optimise the drug:polymer ratio and surfactant concentration. A two component, three level full factorial design was created and carried out in a fully randomised sequence to examine all potential combinations of both factors at all levels.Full factorial design was applied using Minitab software to examine the response surface of a 3-level factorial design with 13 iterations in a quadratic model [16-19]. The 32 complete factorial design used to create the formulations allowed for the simultaneous study of two formulation variables and their interactions. Surfactant concentration and the ratio of medication to polymer were included as two independent variables based on the early research. The comparison of the two variables was done on three levels: +1 (High), 0 (Medium), and -1 (Low). Polynomial equation was used to investigate the impact of the two factors on the responses of the dependent variables, Y1 (Particle Size), Y2 (Entrapment Efficiency), and Y3 (P.D.I).

Table 5: 3^2 Full factorial design runs with actual values of Particle Size, Entrapment efficiency, P.D.I:

Formulation Code	Run	Factor 1 PVA (%)	Factor 2 Drug: EC ratio	Response 1 Particle size (nm)	Response 2 P.D. I	Response 3 Entrapment efficiency (%)
F16	1	0.5	1:0.05	384.80	0.129	78.98
F17	2	0.5	1:0.1	496.20	0.487	65.11
F18	3	0.5	1:0.15	532.80	0.689	54.23
F19	4	1.5	1:0.05	168.30	0.586	81.34
F20	5	1.5	1:0.1	232.40	0.346	72.23
F21	6	1.5	1:0.15	286.80	0.984	69.35
F22	7	2.5	1:0.05	72.04	0.137	84.13
F23	8	2.5	1:0.1	108.40	0.786	79.26
F24	9	2.5	1:0.15	135.50	0.982	73.17
F25	10	2.5	1:0.05	90.18	0.123	83.14
F26	11	2.5	1:0.1	115.6	0.324	74.37
F27	12	2.5	1:0.15	167.9	0.276	78.23
F28	13	2.5	1:0.05	105.34	0.195	80.11

Polynomial equations obtained by 3^2 full factorial designs:

The following polynomial equations for the dependent variables Y1 (Particle Size), Y2 (Entrapment Efficiency), and Y3 (P.D.I.) were created using step-wise backward linear regression analysis and have the form of equation:

$$Y=b0+b1A+b2B+b12\ AB+b11A2+b22\ B2$$

Y is the dependent variable, and the variables b0 and b1 represent the arithmetic means of the responses from the nine batches, respectively. The primary effects (A and B) show the typical outcome of increasing one element at a time from a low value. The answer changes when two factors are adjusted at once, as shown by the interaction term (AB) [20-22]. To study non-linearity, the polynomial terms (A2 and B2) are added. By creating an additional design check point formulation (C), it was possible to confirm the validity of the developed polynomial equations. According to the polynomial equation, the particle size and P.D.I. drop as the surfactant concentration rises, and the drug:polymer ratio also rises, increasing the effectiveness of entrapment.

Regression Equation in Uncoded Units

PS = 369.7 - 140.7 PVA + 1734 EC - 423 PVA*EC

Regression Equation in Uncoded Units

PDI = 0.032 - 0.043 PVA + 3.87 EC + 1.43 PVA*EC

Regression Equation in Uncoded Units

EE% = 88.44 + 0.00 PVA - 254.2 EC + 65.0 PVA*EC

ANOVA for response surface quadratic model of Particle Size, Entrapment Efficiency and P.D.I:

For Particle Size, Entrapment Efficiency and P.D.I, the model F value was found to be 45.96, 46.81, and 4.73 respectively which implies that the model is significant. The R squared value for the model was 0.965, 0.965 and 0.739 respectively; adjusted R squared value was 0.944, 0.945 and 0.583 respectively. Predicted R - square value was 0.906, 0.874 and 0.101 respectively.

Table 6: ANOVA for response surface quadratic model of Particle Size

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Model	3	220815	73605	45.96	0.004	significant
Linear	2	219028	109514	68.38	0.082	
PVA	1	200883	200883	125.43	0.003	
EC	1	18146	18146	11.33	0.020	
2-Way Interactions	1	1787	1787	1.12	0.006	
PVA*EC	1	1787	1787	1.12	0.001	
Error	5	8008	1602			
Total	8	228823				

Table 7: ANOVA for response surface quadratic model of PDI

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Model	3	0.62211	0.20737	4.73	0.004	significant
Linear	2	0.60180	0.30090	6.87	0.037	
PVA	1	0.06000	0.06000	1.37	0.295	
EC	1	0.54180	0.54180	12.36	0.017	
2-Way Interactions	1	0.02031	0.02031	0.46	0.009	
PVA*EC	1	0.02031	0.02031	0.46	0.026	
Error	5	0.21914	0.04383			
Total	8	0.84125				

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value	
Model	3	663.92	221.306	46.81	0.001	significant
Linear	2	621.67	310.833	65.75	0.517	
PVA	1	253.50	253.500	53.62	0.001	
EC	1	368.17	368.167	77.87	0.468	
2-Way Interactions	1	42.25	42.250	8.94	0.030	
PVA*EC	1	42.25	42.250	8.94	0.030	
Error	5	23.64	4.728			
Total	8	687.56				

Table 8: ANOVA for response surface quadratic model of Entrapment Efficiency

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
40.0199	0.965	0.944	0.906

Table 8.1: R squared value of Particle Size

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.209351	0.739	0.583	0.101

Table 9: R squared value of PDI

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.17435	0.965	0.945	0.874

Table 10: R squared value of Entrapment Efficiency

Pareto chart: Data visualisation can reveal response-independent variable relationships. The Pareto graphs of Particle Size, Entrapment efficiency and P.D.I are presented in Figure1, Figure2, Figure3 respectively which illustrates that for Particle Size, Poly Vinyl Alcohol (A) and Ethyl Cellulose (B) showed significant effect. For Entrapment Efficiency, Poly Vinyl Alcohol (A) , Ethyl Cellulose (B) and both Poly Vinyl Alcohol and Ethyl Cellulose (AB) showed significant effect [23-27]. For P.D.I, only Ethyl Cellulose (B) showed significant effect.

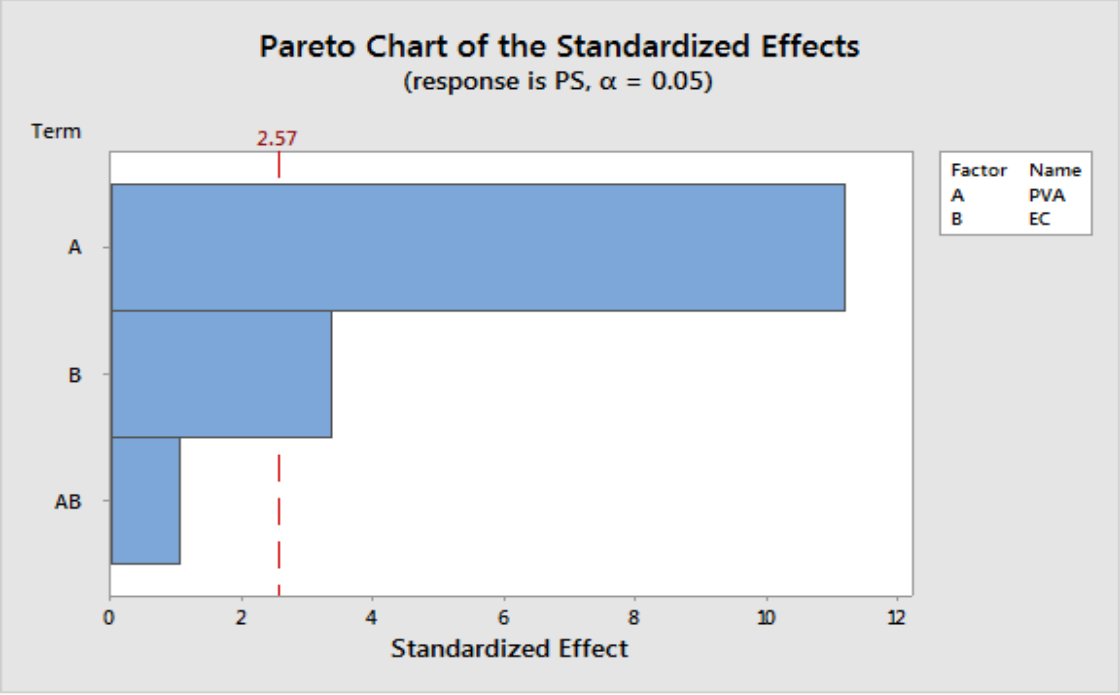

Figure 1: Pareto chart showing the effect on Particle Size

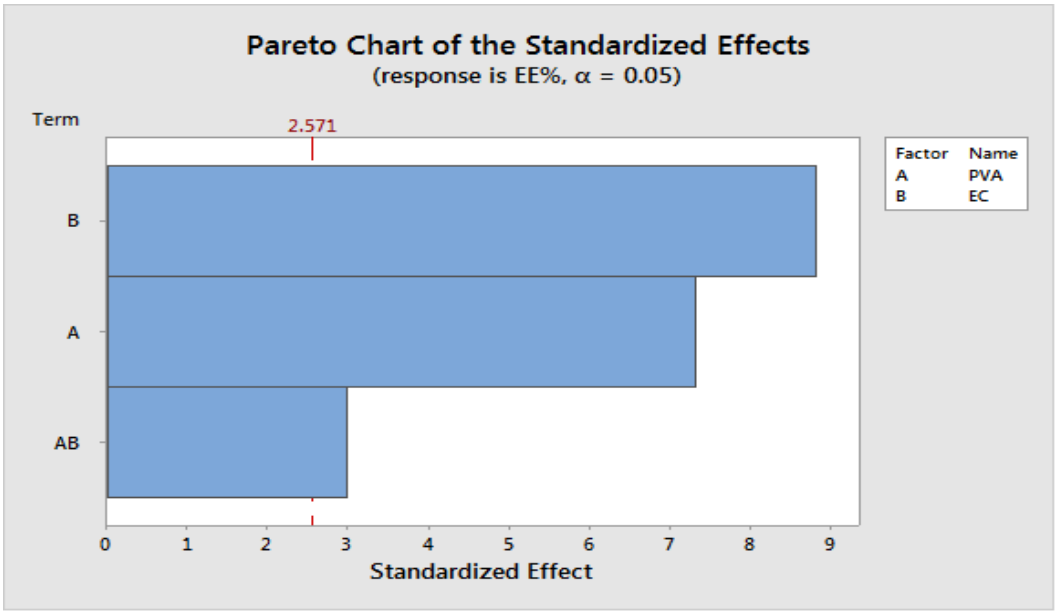

Figure 2: Pareto chart showing the effect on Entrapment Efficiency

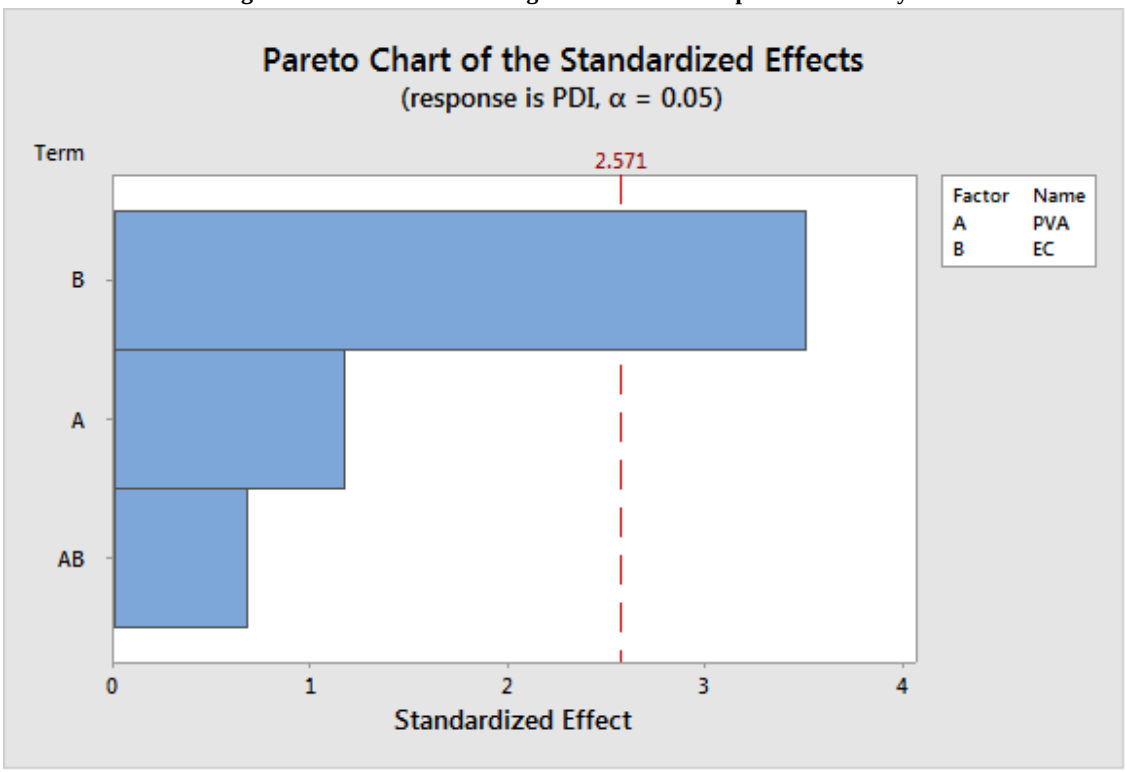

Figure 3: Pareto chart showing the effect on PDI

Surface Counter plot:

The link between the response and the independent variables can be demonstrated graphically. Graphs matched statistical study equations. Figures 4, 5, and 6 show the counter plot graphs for particle size, entrapment efficiency, and P.D.I. An increase in surfactant concentration decreased particle size, increased entrapment efficiency, and decreased polymer concentration [28-30].

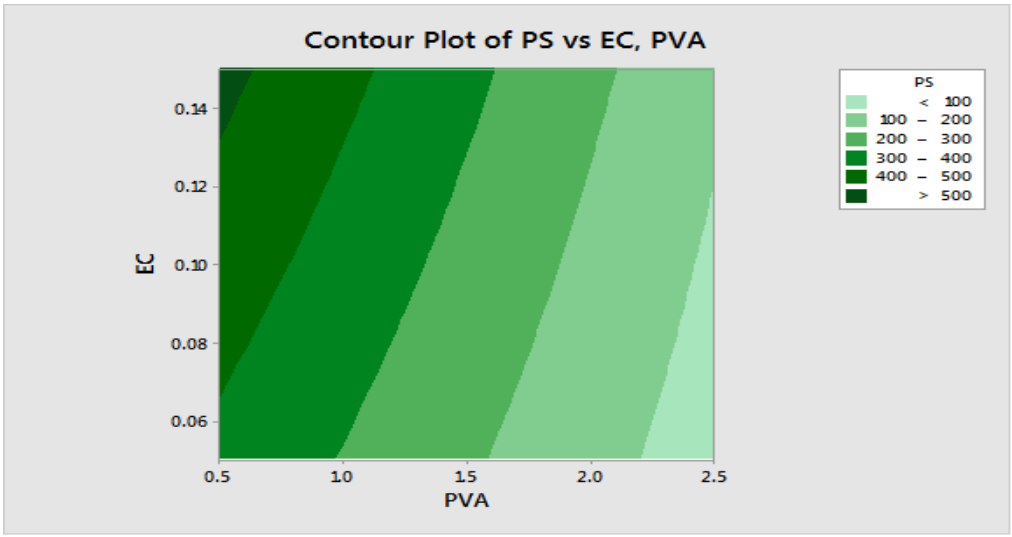

Figure 4: Counter plot (2D) showing the effect on Particle Size

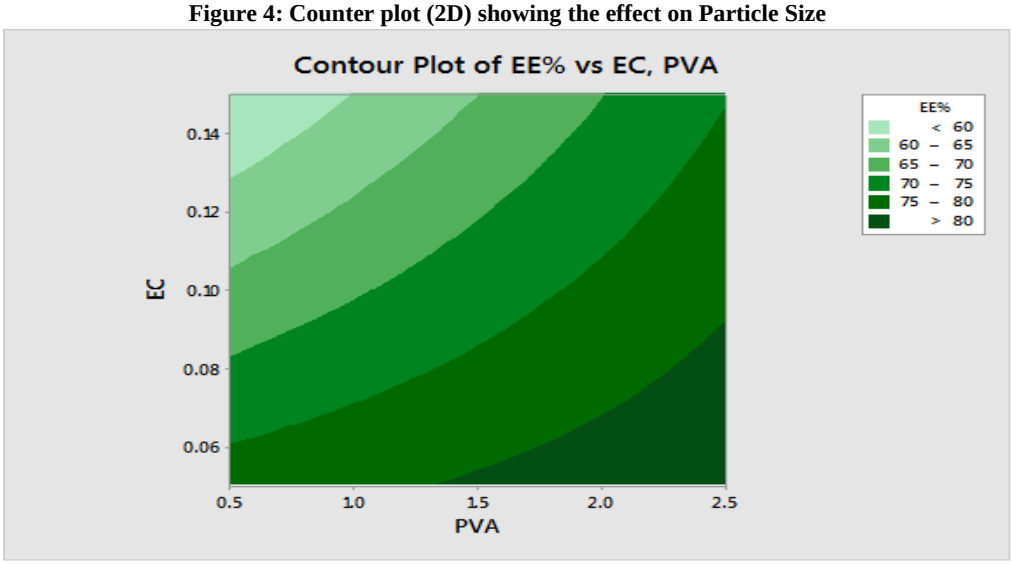

Figure 5: Counter plot (2D) showing the effect on Entrapment Efficiency

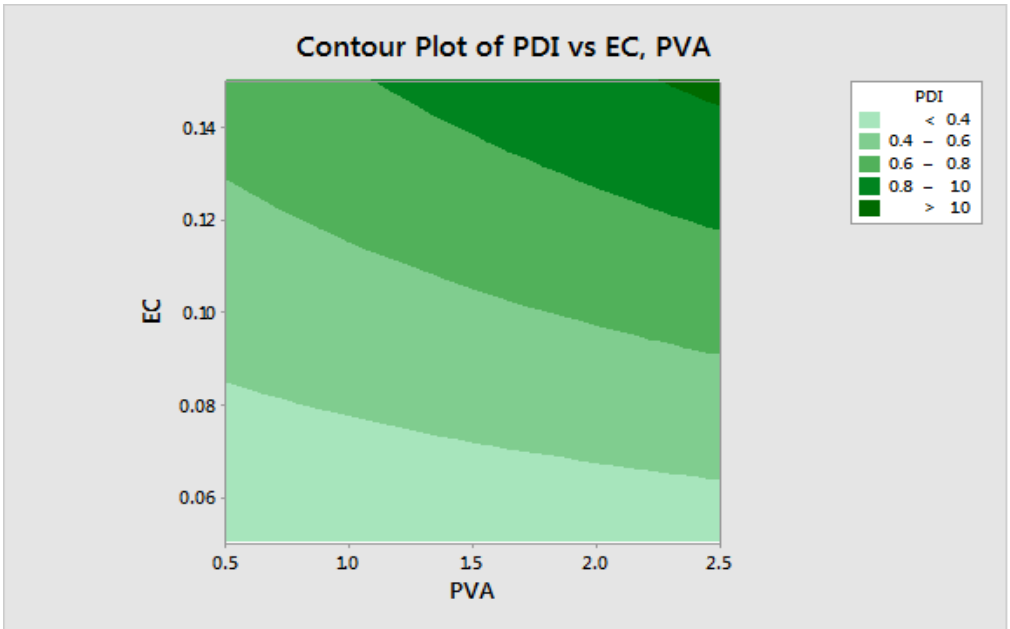

Figure 6: Counter plot (2D) showing the effect on PDI

Response surface plots:

The link between the response and the independent variables can be demonstrated graphically. The information presented in graphs was comparable to that in the mathematical equations derived from the statistical study [31]. Figures 7 through 9 show the response surface graphs for particle size, entrapment efficiency, and P.D.I., which show that an increase in surfactant concentration resulted in a decrease in particle size, a decrease in surfactant concentration, and an increase in polymer concentration, which resulted in an increase in entrapment efficiency, a decrease in surfactant concentration, and a decrease in the concentration of polymer, respectively.

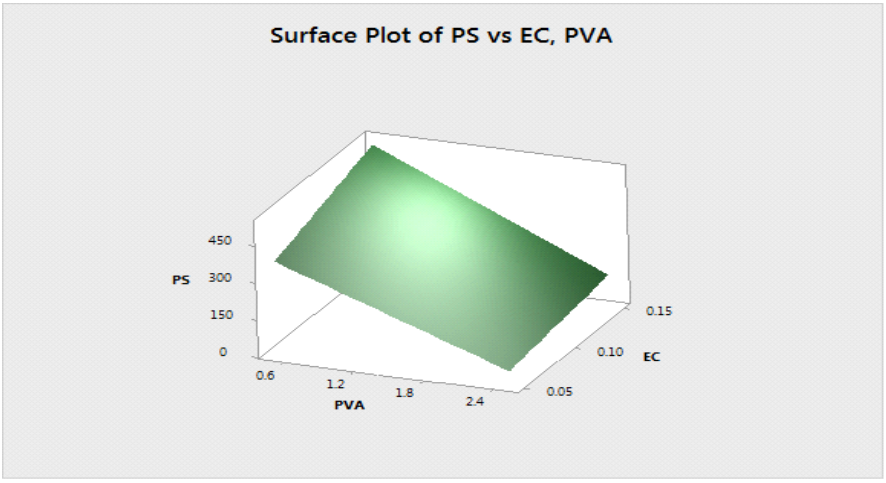

Figure 7: Response surface 3D plot showing effect of factorial variables on Particle Size

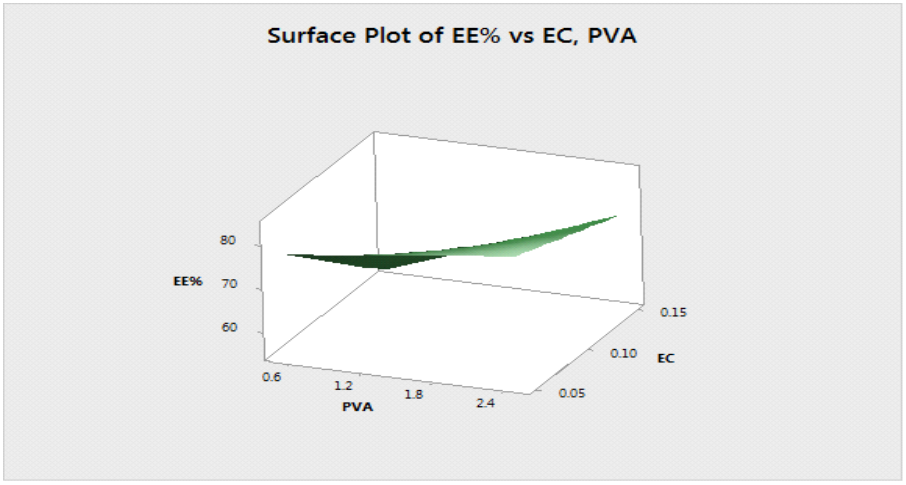

Figure8: Response surface 3D plot showing effect of factorial variables on Entrapment Efficiency

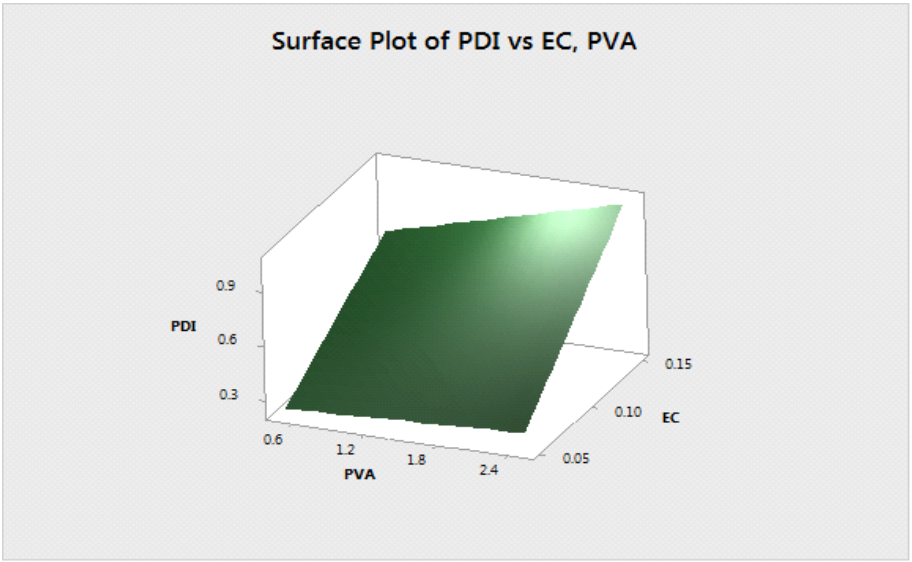

Figure9: Response surface 3D plot showing effect of factorial variables on PDI

Table 11 : 3^2 Full factorial design runs with actual values of Particle Size, Entrapment efficiency, P.D.I: Physicochemical characterization of Clindamycin Phosphate loaded nanosponges

Sl. no	Formulation Code	Particle Size	P.D.I	% Yield	Drug Content	Free Drug	Entrapment Efficiency
1	F16	384.80	0.129	88.90±0.55	84.89±1.28	8.9	78.98
2	F17	496.20	0.487	88.43±1.11	72.46±1.56	14.9	65.11
3	F18	532.80	0.689	85.81±0.67	72.39±2.64	13.09	54.23
4	F19	168.30	0.586	91.82±1.77	85.48±1.99	9.65	81.34
5	F20	232.40	0.346	89.11±1.90	79.38±2.18	17.9	72.23
6	F21	286.80	0.984	87.38±0.50	75.46±1.67	18.0	69.35
7	F22	72.04	0.137	98.76±2.11	88.76±0.58	7.09	84.13
8	F23	108.40	0.786	96.31±0.32	83.29±1.28	9.19	79.26
9	F24	135.50	0.982	94.30±0.55	79.86±2.42	18.9	73.17
10	F25	90.18	0.123	96.89±2.16	85.17±1.04	8.78	83.14
11	F26	115.6	0.324	95.99±0.77	79.02±0.04	8.99	74.37
12	F27	167.9	0.276	91.76±1.34	77.45±1.78	9.73	78.23
13	F28	105.34	0.195	96.54±1.11	82.23±0.04	8.02	80.11

Table 8: Physicochemical properties of prepared nanosponges

Drug Content Analysis:

Different batches of drug-loaded nanosponges were exposed in order to be examined for drug content. The phosphate buffer with a pH of 6.8 was used to dissolve the powdered nanosponges (10 mg equivalent) before filtering. A UV spectrophotometer set at 202 nm was used to gauge the filtrate's UV absorbance. As indicated in table 8 below, the drug concentration of various formulations was discovered to range from 72.461.56 to 88.760.58.

Effective loading of drugs:

Table 8 provides the Clindamycin Phosphate Nanosponge formulations' loading effectiveness (% entrapment). For all nanosponges, the computed loading efficiency ranged from 54.23 to 84.13%. The F22 formulation, in which more medication was encapsulated, had the highest loading efficiency, which was 84%. The more drug was trapped, the higher the loading efficiency.

Yield %:

Table 8 provides the yield percentage for the Clindamycin Phosphate Nanosponge formulation. The computed production yield for all nanosponges ranged from 88.43% to 98.76%. The formulation F22 was found to have the highest production yield, or 98.76%, respectively. The Clindamycin Phosphate Nanosponge formulation production yields showed that the production yield improved as the drug to polymer ratio was raised.

PDI and particle size:

Malvern particle size analyzer was used to perform particle size assessments on all of the formulations. According to PDI, all of the formulated NSs had narrow particle size distributions and were in the nano-size range. F22 exhibited the lowest P.D.I. of 0.137 and the smallest particle size of 72.04 nm (Fig. 3). The average nanosponge particle size depends on the medication-polymer ratio. The low polymer content promotes dichloromethane (internal phase) diffusion into aqueous phase (external phase), reducing droplet generation time and particle size. The F1 formulation used in the Preformulation experiments is the same as the F22 formulation.

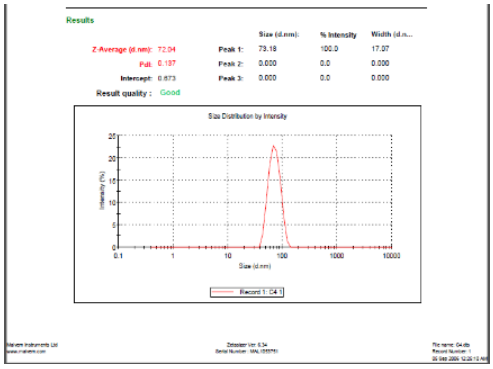

Figure10: Particle Size and PDI of F22 Formulation

Scanning electron microscopy:

SEM was used to examine the morphology of the optimised Nanosponge created using the F22 emulsion solvent diffusion method. Figure 4 depicts representative SEM images of the nanosponges, which illustrate their spherical form and range of nanosizes. It was hypothesised that the porous, spongy quality of the nanosponges was a result of the inward diffusion of DCM on the EC polymeric surface. The micrographs (Figure 11) further showed that the clindamycin phosphate was appropriately coated on the EC matrix, which was then glazed by PVA to prevent adhesion between the particles and the smooth surface of the Nanosponges.

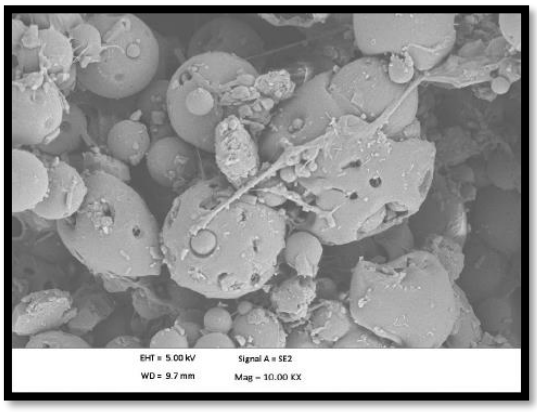

Figure11: SEM of Optimized Formulation

Franz diffusion cells with a 38 ml receptor cell volume and a 5 ml donor compartment were used for the diffusion studies. The phosphate buffered pH 7.4 used to imitate the physiological pH in the receptor compartments. For 24 hours, the % release of the two optimised formulations (F22 & F25) was investigated. When compared to other formulations, it was shown that F28, which contains polymer and retardant material in a 1:0.5 ratio, has the highest level of in vitro drug release. The maximum drug release, or 83.5%, was seen with F22. this might be related to the highest level of drug encapsulation in the NS's inner structure. Figure 6 displays the F22 and F23 comparative drug release investigations.

Additionally, various mathematical models of release kinetics have been applied to the release profiles of all formulations; As indicated in Table 10, the interpretation of the drug's data revealed that the Higuchi classical model of diffusion produced the maximum linearity (highest R2 value). Drug release occurs via diffusion. The Korsmeyer-Peppas model was applied to 60% of release data to determine if this diffusion mechanism is Fickian. The Korsmeyer-Peppas model was the best fit for F22 with a regression coefficient (r2) of 0.997, indicating that the exponent (0.45 n 0.89) 'n' values are less than 0.5, indicating that Fick's law of diffusion is being followed. According to the Korsmeyer-Peppas model, all nanosponges were non-swellable matrix diffusion drug release mechanisms due to formulation porosity. F22 showed the highest EE and in vitro drug release. This formulation underwent further characterization.

Table 12: % Drug Release studies of the nanosponges

SL. NO	TIME	%CDR	
		F22	F25
1	0	0	0
2	0.5	5.87 ± 1.28	4.36 ± 0.12
3	1	10.76 ± 1.21	11.32 ± 0.21
4	2	20.68 ± 0.19	23.05 ± 0.14
5	3	33.41 ± 0.12	30.56 ± 0.16
6	4	39.86 ± 0.09	37.12 ± 0.16
7	5	43.34 ± 0.11	45.13 ± 0.26
8	6	50.86 ± 0.34	54.83 ± 0.24
9	7	65.89 ± 0.12	63.92 ± 0.20
10	**24**	**83.5 ± 0.12**	77.7 ± 0.21

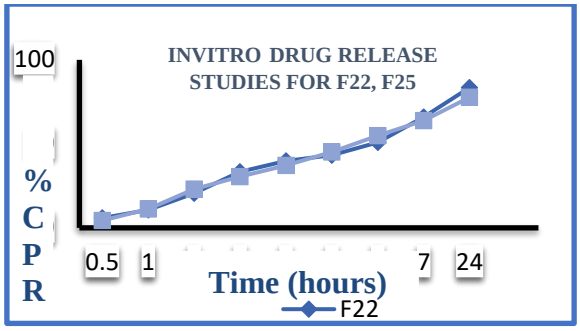

Figure12: Comparison Drug release studies of F22, F23

Table 13: Kinetic data model release for nanosponges

Code	Zero order		First order		Korsmeyer Peppas		Matrix		Best fit model
F22	R^2	K	R^2	K	**R^2**	**K**	R^2	K	-
	0.984	2.536	0.959	2.273	**0.997**	**0.435**	0.977	3.412	**KorsmeyerPeppas**

Gel loaded Nanosponge

For further inclusion into a topical gel, the F22 Nanosponge formulation was chosen based on the particle size, entrapment, and release. Carbopol 934 was used to create gels as the gelling agent. It was discovered that 1% Carbopol 934 worked best for producing an efficient andsatisfying gel. A non-consistent gel was produced with lower (0.5%) or higher (1.5%)amounts of carbopol. The formulations G5 and G6, which included Permeation Enhancers,were chosen because our goal was to achieve increased permeability. The hydrogels'physicochemical characteristics were assessed.

Homogeneity:

The homogeneity of gel refers to its uniformity and consistency. It is necessary to preserve homogeneous nature. The synthesised gel formulations G5 and G6 were discovered to be homogeneous and transparent. It didn't leave a scratchy feeling on the skin's surface after application.

pH:

In gel formulations, the pH is kept constant to mimic the skin's pH balance. For topical usage, a gel's optimal pH range is 4.5 to 7. If the gel's pH rises above 7.2, it enters an alkaline condition and begins to irritate the skin. The produced gel formulations G5 & G6 were found to have a pH between 6.52 and 6.79.

Viscosity:

The optimal viscosity of gels ranges from 2000 to 6000cps, depending on the viscosity of the polymer. The stability of gels is impacted by the gel's viscosity. Gel loses its ability to spread and exhibits poor extrudability as its viscosity rises. Using a Brooke field viscometer, the viscosity of the produced gel compositions G5 and G6 was assessed. Their viscosity ranged from 4500 cps to 5356 cps.

Spreadability:

Spreadability is a term used to describe how far the gel will spread when applied to skin or an afflicted area. The spread ability parameters of the formation also influence how effective it is as a treatment. As a result, measuring spreadability is important for evaluating gel properties. The spreadability of the manufactured gel formulations G5 and G6 was assessed, and the findings revealed that spreadability ranged from 8.28 gm/sec to 10.56 gm/sec, respectively.

Extrudability:

This common empirical test determines the amount of force required to extrude a material from a tube. The extrudability of the produced gel formulations G5 and G6 was assessed. Good Extrudability behaviour was displayed by G6.

Drug content:

The amount of drugs in the manufactured gel formulations G5 and G6 was assessed. G5 was found to contain 85.79% of drugs, while G6 contained 91.8%.

Table 14: Evaluation of standardized Nanosponge gels

Sl. No	1	2
Formulation code	G5	G6
Appearance and homogeneity	Gel like consistency, very good homogeneity, Translucent	Gel like consistency, very good homogeneity, Translucent
pH ± SD	6.52	6.79

Viscosity ± SD (cps)	4500 ± 0.2449	5356 ± 0.1476
Spreadability (gm.cm/sec)	8.28	10.56
Extrudability	Good flow	Excellent flow
% Yield ± SD	92.63	93.63
Drug content (%) ± SD	85.79 ± 0.0147	91.8 ± 0.0121

***In-vitro* release studies of gels:**

For 24 hours, the percentage releases of the standardised Nanosponge gel G5 & G6 were investigated. The gels demonstrated sustained release over a 24-hour period. % Release for the first and seventh hours, as well as the seventh and twenty-fourth hours, were respectively 9.39% and 10.48%, 64.86% and 70.89%, and 84.45% and 88.96% (Table 12 & Fig 7).

Table 15: *In Vitro* Release Study of the Formulated Nanosponge loaded Gels

Sl.no	Time	%CDR	
		G5	G6
1	0	0	0
2	1	9.39 ± 0.27	10.48 ± 0.20
3	2	12.31 ± 0.098	14.38 ± 0.3710
4	3	22.36 ± 0.231	23.92 ± 0.102
5	4	36.1 ± 0.231	35.89 ± 0.278
6	5	45.47 ± 0.156	50.38 ± 0.2298
7	6	53.13 ± 0.121	66.1 ± 0.288
8	7	64.86 ± 0.202	70.89 ± 0.222
9	24	82.45 ± 0.512	88.96 ± 0.212

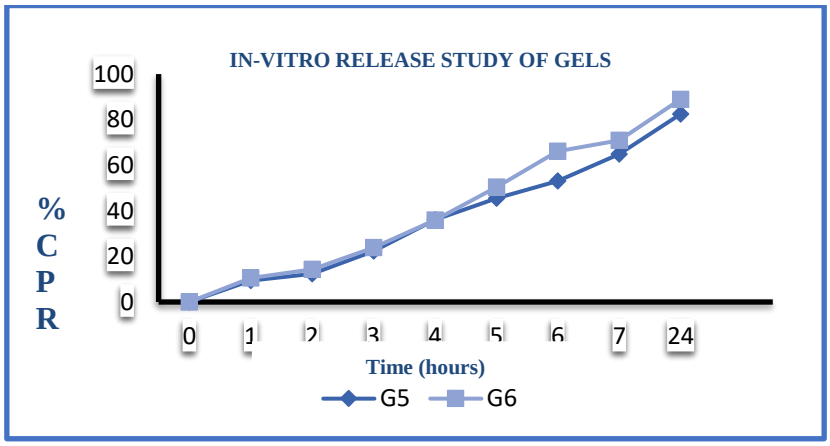

Figure13: In vitro release study of gels

4.Conclusion

Lincosamide as a medication Based on novel Carbopol 934 gels, loaded Nanosponge loaded hydrogel was effectively created using DOE using Minitab® Programme as screening and optimisation phases. Optimisation is a sophisticated experimental procedure, similar to those employed in the development of nanoparticles, that results in reliable preparation methods and a product with the desired qualities. The most popular technique for optimising studies and determining which variables dominate the result and at what level they serve as a guide for a better and desirable Dosage Form is the use of factorial designs. Factorial analysis revealed that formulation F22 was superior to other formulations and was therefore subjected to additional analysis.

The optimal formulation was then determined to be the chosen antibiotic medication, Clindamycin Phosphate developed into Nanosponge, which was distributed into Carbopol 934 gel for topical treatment as a unique technique.

Author contribution

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission

Conflict of interest

The authors declare that no conflict of interest is associated with this work.

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