Section A-Research paper

FORMULATION AND EVALUATION OF VALACYCLOVIR NIOSOMES BY BOX–BEHNKEN EXPERIMENT DESIGN

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ABSTRACT

Objective: The present research work was to formulate and evaluate Valacyclovir by using Box-Behnken Design, the current study intends to create and assess valacyclovir niosomes.
Method: By employing the non-ionic surfactant Brij 72, the charge inducer diacetyl phosphate and cholesterol, stable Valacyclovir-loaded niosomes can be created. To study and optimize the primary effects, interaction effects and quadratic effects of the formulation ingredients on the performance of the niosomes, a total of 17 (VF1-VF17) formulations with the above concentrations was prepared using a three-factor, three-level Box-Behnken design. **Results:** Preformulation studies and drug excipients compatibility studies was done initially and results directed the further course of formulation. Various evaluation tests such as Zeta potential, Drug Content, Polydispersity Index, Entrapment Efficiency and Cumulative percent of drug released were performed to optimize the formulation. Most of the vesicles are spherical in shape, the size range of the vesicles fall in the narrow size range. A high % of valacyclovir can be encapsulated in the vesicles (72-86%) prepared by thin film hydration technique. Concentration of non-ionic surfactant and charge inducer might influence the drug release pattern of all formulation. *In-vitro* release of valacyclovir from niosomes was very slow and maintained prolong release, when compared to the release from pure valacyclovir solution. The Cumulative percent drug released of all prepared formulation shown (VF1-VF17) between 78.43% - 98.91%. Among the all formulations VF13 had shown 98.91% of drug release at 24 hrs.

Conclusion: The niosomal formulation was stable, according to drug release experiments. Based upon all the evaluation tests, the formulation VF13 was considered as optimized. In all formulations, drug release was observed in all formulations indicating zero order release pattern.

KEYWORDS: Valacyclovir, Niosome, Thin Film Hydration Technique.

INTRODUCTION

to liposomes and were developed as an alternative delivery system to liposomes, as niosomes can overcome the problems associated with large-scale production, sterilization and physical stability. Niosomes are novel vesicular Drug delivery system by which we can achieve the constant plasma drug concentration for the extended period of time[1] Niosomes are formed by self-assembly of non-ionic surfactants. They are structurally similar. To increase the stomach absorption of compounds with restricted permeability, researchers have employed a variety of cutting-edge approaches, including prodrugs, microemulsions, liposomes, niosomes, bilosomes, and others. Because they act as drug reservoirs and can have their composition adjusted to manage the pace of drug release, vesicles have been found to have considerable advantages over other techniques. Depending on the technique employed for their preparation, they may be unilamellar or multilamellar[2].The antiviral action of valacyclovir prevents virus replication. It undergoes transformation into acyclovir and ultimately into acyclovir triphosphate (ACV-TP). Viral DNA polymerase is competitively inhibited by ACV-TP, which integrates into and breaks the growing viral DNA chain. Viral DNA is therefore inactivated. It is known that long-term antiretroviral therapy utilizing a higher dosage regimen reduces necessary to both and maintain viral suppression. The unusually short biological half-life of valacyclovir is the key factor limiting its therapeutic effectiveness3. Large doses must be administered frequently due to this. Because it's essential to keep the systemic drug concentration at a therapeutic level throughout the course of treatment. Niosomes were chosen as the carrier in an efficient drug delivery system to distribute valacyclovir in order to address these two drawbacks. This allows for a better degree of targeting of medications to certain tissues in a regulated manner[4]. Niosomes made from valacyclovir were generated in the current study and tested for their in vitro properties in an effort to increase the drug's oral bioavailability and lengthen its release time[5]**.**

MATERIALS AND METHODS

Materials

Valacyclovir was given away as a sample by Bangalore's Micro Labs. We bought Brij 72, diacetyl phosphate, chloroform, and cholesterol (CHOL) from SD Fine Pvt. Ltd. in Hyderabad. All additional compounds were of the analytical variety and were bought from reliable suppliers.

Niosomal Formulation Preparation

The thin film hydration approach was used in the current investigation to create niosomal formulations. The primary impacts, interaction effects, and quadratic effects of the formulation ingredients on the functionality of the niosomes were investigated and optimized using a three-factor, three-level Box-Behnken Design. The examination of quadratic response surfaces and the building of second-order polynomial models are both made possible by this architecture. The three-factor, three-level BBD was used to create the 17 randomized experimental runs for the chosen independent variables, five of which included replicates at the center (asterisk-marked). In order to more precisely calculate the prediction variance across the whole design space, five replicates at the center point were used in this experiment. The mixture was gradually being dosed with medication. The amount of membrane stabilizer, surfactant, and charge inducer were classified as low (coded as +1), intermediate (coded as 0), and high (coded as +1) degrees of independent or formulation

variables based on the boundary of the niosomes domain. Cholesterol, which stabilizes membranes, Brij 72, a surfactant, and diacetyl phosphate, an inducer of charges, were selected as the ranges for each independent variable for niosomes, and they were determined to be between 30 and 50 percent, 20 to 40 percent, and 2 to 6 percent, respectively (Table 1).. In order to evaluate the quality of the Niosomes formulation, three important response factors—particle size (Y1), entrapment efficacy (Y2), and cumulative percentage of drug released (Y3)—were identified. (Table 1)

Table 1. List of dependent and independent variables in in Box-Behnken design

Independent variables			***Levels***		
Variable	**Name**	**Units**	**Low (-1)**	**Middle (0)**	**High (+1)**
A	Cholesterol	%	30	40	50
B	Brij 72	%	20	30	40
C	Diacetyl Phosphate	%	2	4	6
Dependent variable			***Goal***		
Y1	Particle size	nm	Minimize		
Y2	Entrapment Efficiency	%	Minimize		
Y3	Drug release after 24 Hrs	%	Maximize		

Exact weighted amounts of the surfactants and cholesterol were acquired, dissolved in 10 ml of chloroform in a round-bottomed flask, and then DCP was added to obtain the proper ratio. After that, the medication was precisely weighed and added to the solvent. After the solvent was evaporated in a rotary flash evaporator at 60^0C and 120 rpm under a vacuum of 20 inches of Hg, a smooth, dry lipid layer was produced. The solvent was then delivered under intense vacuum for at least three hours through a vacuum pump in order to remove any leftover chloroform content. A second flask was left in vacuum desiccators overnight to ensure that all of the chloroform was removed. After that, the film was hydrated for 3 hours at 60 degrees Celsius by being mixed in 10 ml of PBS pH 6.8. The niosomal suspension was maintained for 24 hours at 2-8 0C. The stability tests, zeta potential, polydispersity index, in vitro drug release profile, particle size, shape, and entrapment effectiveness of the produced niosomal formulation were assessed. In 10 ml, the aqueous phase was sampled. 3 hours and 60 degrees were the appropriate conditions for hydration. These preparations underwent size distribution and entrapment efficiency improvements[7].

Table 2 Composition of valacyclovir niosome formulation by Box-Behnken Design

F.No	**Valacyclovir (mg)**	**Cholesterol (%)**	**Brij 72 (%)**	**Diacetyl Phosphate (%)**	**Chloroform (ml)**	**Distilled Water (mL)**
VF1	500	30	20	4	10	Q.S
VF2	500	50	20	4	10	Q.S
VF3	500	30	40	6	10	Q.S

VF4	500	40	40	2	10	Q.S
VF5	500	30	40	2	10	Q.S
VF6	500	50	20	2	10	Q.S
VF7	500	30	30	6	10	Q.S
VF8	500	50	30	6	10	Q.S
VF9	500	40	20	2	10	Q.S
VF10	500	50	40	4	10	Q.S
VF11	500	40	20	6	10	Q.S
VF12	500	40	40	4	10	Q.S
VF13	500	50	40	6	10	Q.S
VF14	500	40	30	2	10	Q.S
VF15	500	40	40	6	10	Q.S
VF16	500	30	30	4	10	Q.S
VF17	500	40	20	4	10	Q.S

Characterization of niosomes

Size and Zeta potential

Using the Malvern zeta sizer, version 7.11, Zeta potential, size, and polydispersity index (PDI) were computed. It provides a report on size distribution by intensity8. In Table 2, it is displayed.

Morphological features

To analyse the morphology and shape, a field emission & SEM was employed. A drop of the niosomal formulation is applied on an aluminum stub using silver adhesive tape. Aluminum stubs were placed in a vacuum overnight, followed by a gold-based sputter coating technique[9].

Analysis of FTIR

FTIR was used to conduct investigations on the compatibility of drugs and excipients. Using the potassium bromide (KBr) disc technique, the spectra of pure medication (valacyclovir), Span, Tween, Brij, and cholesterol as well as their physical combination were captured. The spectrum of infrared light was measured between 500 and 4000 cm-1. Figures 2 and 3 depict the IR spectra of a pure medication, a surfactant, cholesterol, and a combination.

Drug content

Niosome valacyclovir concentration was measured using a UV spectrophotometric technique. Niosomes were dissolved in 10 ml of methanol after being dosed with 10 mg of the medication equivalent. A UV spectrophotometer was used to measure the absorbance after the appropriate dilution in order to ascertain the drug content at a maximum 254 nm against a blank. Table 2 displays the valacyclovir niosomes' drug content

Vesicle Size Measurement

The optical microscope (Vaiseshika 7001-IMS) was used to assess the average vesicle size of the manufactured niosomes. Then, 100 randomly selected niosome vesicles from each formulation were measured for size in vesicle size distribution experiments on the optimised

batches. To examine the vesicles' structure at higher magnification levels, the Scanning Electron Microscopy (SEM) technique was used[10].

Drug Entrapment efficiency of niosomes

The effectiveness of niosome entrapment was assessed using an extensive dialysis approach. Niosomal suspension in the appropriate amount was placed in a dialysis tube with an osmosis cellulose membrane firmly attached to one side. A magnetic stirrer was used to agitate 100 ml of buffer contained phosphate was used. Through osmosis cellulose membrane, the unentrapped medication was removed from the niosomal solution and introduced into the medium. Since no medication is present in an unentrapped form, the complete medium (100ml) was changed out every hour until the absorbance attained a consistent reading. The dialysis tube's niosomal solution was further lysed with propane-1-ol, and the amount of drug entrapped was determined using a UV spectrophotometric technique at 254 nm. Equation 11 was used to calculate the entrapment efficiency[11].

$$\textbf{Entrapment Efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug}} \times 100$$

Drug release studies (*In vitro*)

An adaptation of the Franz diffusion cell method was used to measure the release of valacyclovir from niosomal solution, 100 mg equivalent valacyclovir-loaded niosomal solution was deposited. To fill the compartmental receptor PBS was used, which was maintained at 37^{o}C and magnetically spun at 50 rpm. The valacyclovir content was evaluated by sampling 1mL of receptor fluid at preset intervals. The withdrew amount was substituted with an equal amount of freshly made buffer, and UV spectrophotometric measurements were taken at 254 nm (Jasco, Model UV-2400, Japan) [12].

RESULT AND DISCUSSION

It was preferable to distribute Valacyclovir-loaded niosomes because they would increase the residence duration of drug delivery and allow for effective absorption of the active component. Niosomes were chosen for the current investigation since it was claimed that they are the simplest method from a technical and logical standpoint among extended drug delivery systems.

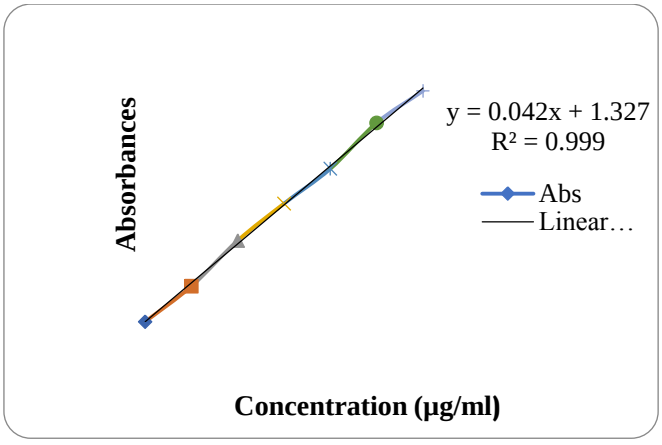

Figure 1. Standard graph of valacyclovir in phosphate buffer 6.8

Standard graph of valacyclovir in phosphate buffer 6.8 was performed to know the slope value (0.42), it is used to calculate Cumulative percent of drug release.

$$\text{Slope} = \frac{Y_2 - Y_1}{X_2 - X_1}$$

FTIR Studies:

On drug, excipient, and drug-excipient samples, FTIR experiments were conducted. Since no additional peaks were discovered, it was determined that the drug and the excipients were compatible.

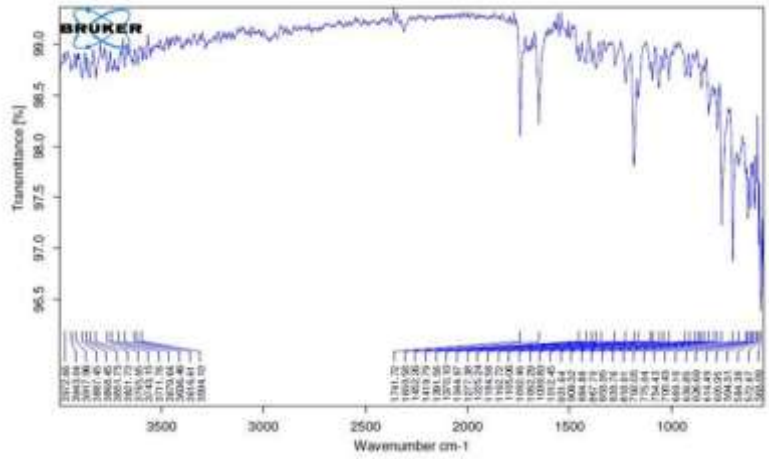

Figure 2 FTIR interpretation of Valacyclovir

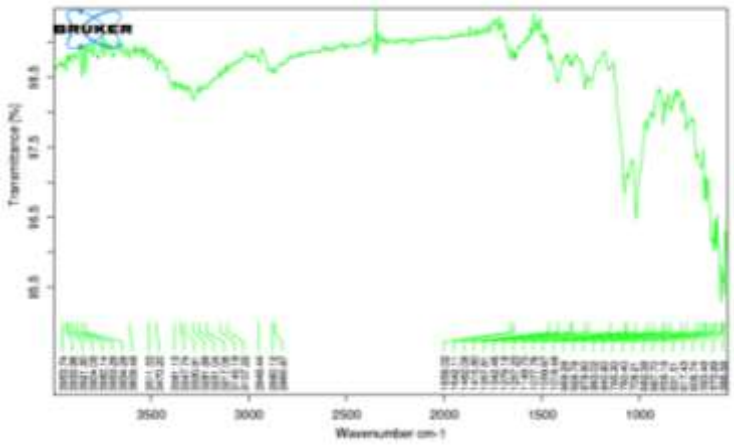

Figure 3 FTIR interpretation of Valacyclovir (VF13) optimization formulation

Scanning Electron Microscopy (SEM) technique

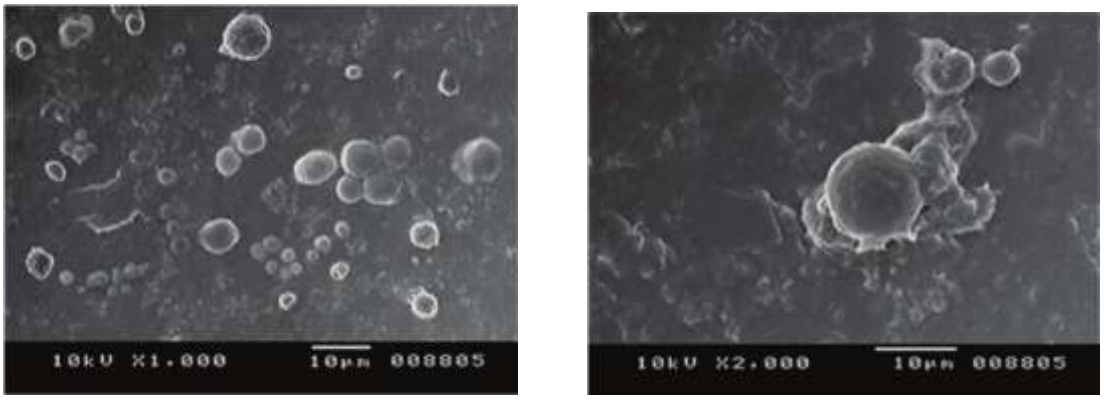

Figure 4 (a) SEM image of valacyclovir loaded niosomes, (b) spherical structure of niosome at higher magnification.

Zeta Potential Determination

VF1-VF17 formulations were produced high and showed no statistically significant differences. The zeta potential's absolute value serves as a measure of the stability of niosomes, the higher the zeta potential's absolute value, the more surface charge there is, which results in more repulsive interactions and more stable. Additionally, utilising cholesterol as a membrane stabiliser makes the vesicular bilayer more rigid and reduces the amount of drug released from the niosomal system, which improves niosome stability.

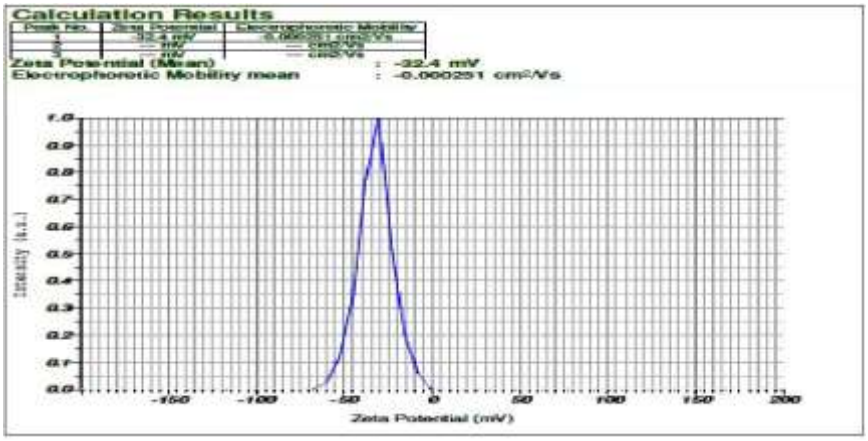

Figure 5 A typical graph for zeta potential of VF13 batch

Niosomes' physical characteristics were evaluated, and the results are shown in Table. As shown in Table, our results showed PDI values ranging from 0.325 to 0.424, indicating variance in the particle size distribution across all formulations. Brij 72, which has a high concentration, has the highest PDI, which indicates the highest homogeneity in the niosomal dispersion, whereas span looks at the highest value of the PDI, which is 0.424 and indicates a heterogeneous niosomal system.

The results revealed that the zeta values of the vesicles increase toward negative with increasing the HLB values of the surfactants. The effect of HLB values of surfactants on zeta potential could be explained in terms of surface energy, which tends to increase with increase in HLB values toward the hydrophilicity. Increase in surface energy of the vesicles leads to

increase the values of zeta potential toward negative. The high negative surface charge on niosomes indicates higher stability because of the anticipated surface repulsion between similarly charged particles therefore, inhibiting aggregation of the colloidal niosomal particles. It was observed that all the formulations were sufficient to keep the particles stable.

Table 3. The evaluation test of entrapment efficiency, content uniformity, % CDR, polydispersibility index and zeta potential for all the batches (VF1-VF17).

F.No	# Content uniformity (%)*	% Entrapment Efficiency*	Cumulative % Drug Released*	Zeta potential (-Mv)± SD*	Polydispersibility index
VF1	97.45±1.26	78.9±1.46	83.12±1.52	-24.84 ± 0.79	0.389
VF2	96.33±2.89	75.4±1.57	85.34±0.52	-20.29 ± 1.03	0.420
VF3	95.12±2.43	78.5±1.65	78.88±1.16	-25.44 ± 0.92	0.385
VF4	97.18±1.52	78.8±2.36	78.43±0.32	-21.07 ± 1.75	0.325
VF5	97.51±2.16	81.7±1.19	81.34±0.25	-24.57 ± 0.16	0.370
VF6	97.73±2.33	73.5±1.54	83.55±0.29	-25.69 ± 1.87	0.387
VF7	98.44±2.67	76.6±2.88	79.66±1.86	-28.27 ± 0.35	0.404
VF8	97.23±2.89	83.8±1.72	91.67±2.21	-30.55 ± 0.68	0.395
VF9	96.11±2.53	77.7±1.57	82.49±2.89	-29.40 ± 0.21	0.376
VF10	98.78±2.54	81.4±2.35	94.77±0.85	-18.71 ± 1.43	0.385
VF11	96.88±2.65	77.4±1.23	87.15±1.66	-22.89 ± 1.62	0.334
VF12	95.23±1.62	73.2±2.91	86.79±0.88	-26.47 ± 0.19	0.353
VF13	**99.56±1.89**	**86.4±.2.84**	**98.91±2.81**	**-32.82 ± 1.11**	**0.424**
VF14	97.01±2.39	81.2±1.70	80.62±1.74	-21.44 ± 1.78	0.351
VF15	96.78±2.17	74.2±2.67	88.25±1.14	-25.96 ± 0.35	0.417
VF16	96.45±2.56	79.6±1.42	80.15±0.85	-29.58 ± 1.23	0.387
VF17	97.26±2.13	73.8±2.18	93.49±1.11	-24.44 ± 0.88	0.373

* Mean ± Standard Deviation; (n=3)

By the above results the drug content of all prepared formulation (VF1-VF17) between 95.12% - 99.56%. It clearly mentions that all the formulations contain uniform quantity of drug. The % Entrapment Efficiency of all prepared formulation (VF1-VF17) between 73.2 % - 86.4% it denotes that all the formulations effectively entrapped the drug. The Zeta potential of all prepared formulation (VF1-VF17) between -18.71 to -32.82, it helps to explain stability of formulations. Among the all formulations VF13 is more stable. The Cumulative % Drug released of all prepared formulation (VF1-VF17) between 78.43 - 98.91%. Among the all formulations VF13 had shown 98.91% of drug release at 24 hrs. It is an important evaluation parameter to select an optimize formula. The Polydispersibility index of all prepared

formulation (VF1-VF17) between 0.325 - 0.424. It is also useful evaluation test to know the stability of formulations.

In vitro Drug Released studies

Following the prescribed protocol, the drug release pattern was examined for 24 hours for all formulations (VF1 to VF17). The results are shown in Figures 6, 7, and 8. The kind and proportion of nonionic surfactant and cholesterol in niosomes affected how the drugs were released from them. The drug & polymer ratio has showed significant mark on the drug release pattern pf the niosomes. The formulation VF13 was found to be the best with a high percentage of release of drug, according to the total data on the in vitro dissolution trials.

From results, it is obvious that the increase of cholesterol molar ratio reduced the efflux of the drug from niosomal preparations, which is in accordance with its membrane-stabilizing ability. Cholesterol is known to abolish the gel to liquid-phase transition of niosome systems, resulting in niosomes that are less leaky. Therefore, the diffusion of pilocarpine HCl entrapped in the hydrophobic regions of the vesicles would be expected to occur over a prolonged period of time. It was observed that viscosity of all the formulations was decreasing with the increase in shear rate. The non-Newtonian formulations with pseudoplastic properties can acquire a viscosity decrease with increasing shear rate

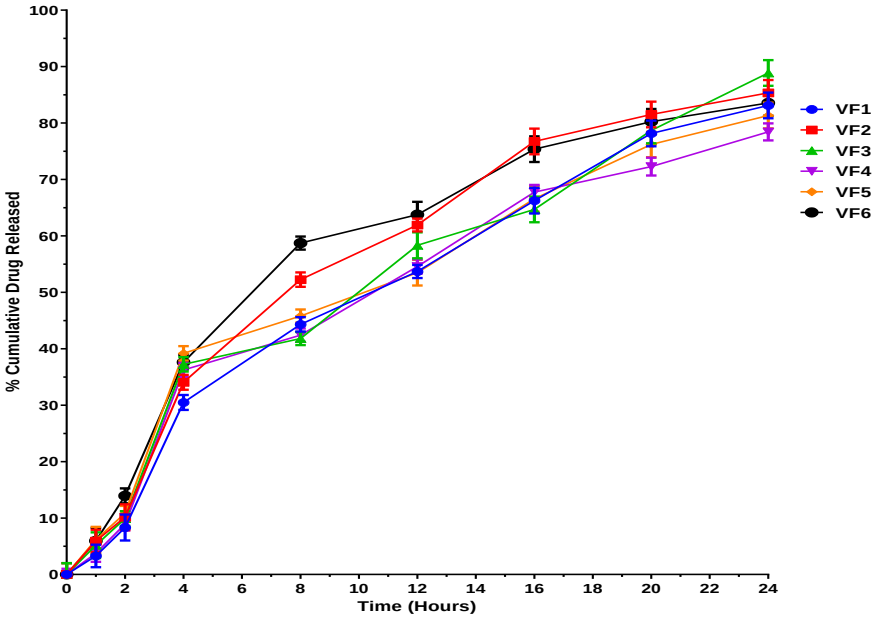

Figure 6. *Invitro* Drug Released Profile of valacyclovir niosomes of formulations VF1-VF6

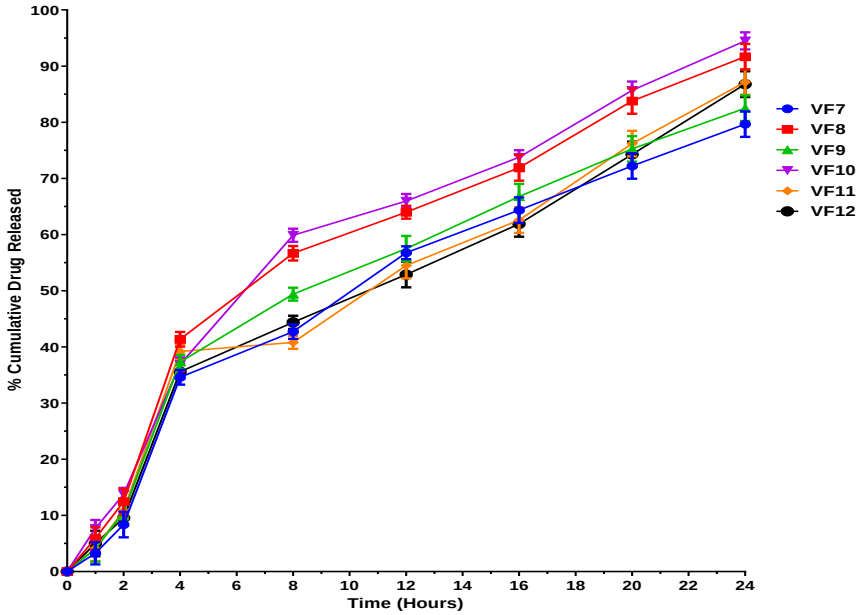

Figure 7. *Invitro* Drug Released Profile of valacyclovir niosomes of formulations VF7-VF12

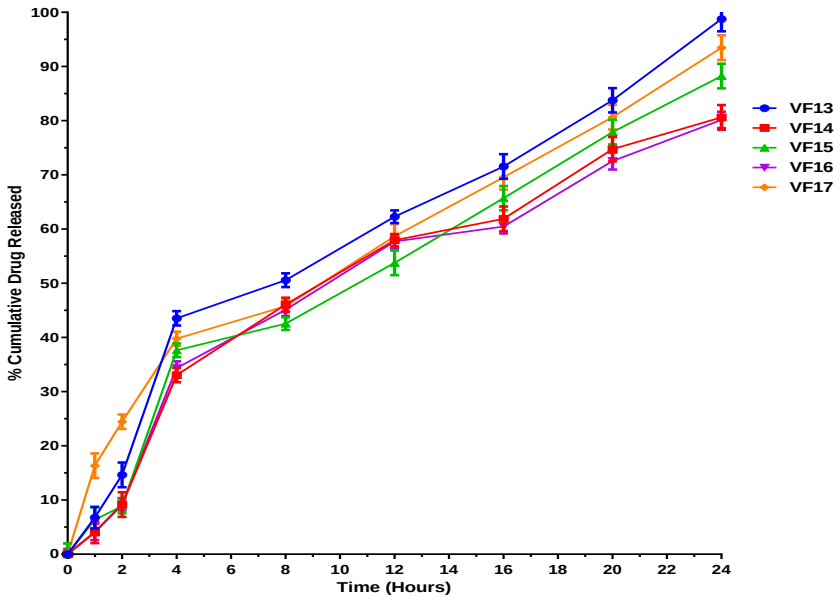

Figure 8 *Invitro* Drug Released Profile of valacyclovir niosomes of formulations VF13-VF17

Analysis of statistics by DoE

Based on the experimental data, several tests were conducted by 3^3 factorial designs with BBD. Observed and independent outcomes for the randomized trials were shown in

Table.1.For all the batches the Polydispersibility index is 0.325-0.424. The range of percent entrapment efficiency (Y2) was 73.2 -86.4% with 24h of cumulative drug release was 78.43-98.91%. The appropriateness of the model was evaluated by ANOVA, the findings were fitted with quadratic second model and were fitted to each of the three responses separately. Results from models were independently verified by using ANOVA. All the responses performed with quadratic model of second order which generated the highest value of F were considered as suitable model. A great statistical characteristic for assessing the model's fitness is the absence of a fit test. Quadratic mode with the correctness was shown by the ability to match all of the replies with the significant lack-of-fit F value ($p>0.5$). The R2 value, a gauge of how much variation around the mean the model is able to account for, is also used to illustrate the multiple regression analysis for the second order quadratic model.

Determination of the second order model

For estimation of coefficients in the approximating polynomial function applying coded values of factor levels, the least square regression method was performed using the SAS System statistical software. By applying regression analysis methods, the predicted responses have been obtained. The resultant equations are shown in Table….

Table 4 : Regression Equations of the fitted models

Response	Equation
Particle Size (Y_1)	$189 +24 X_1 - 19 X_2 - 10 X_3 -16X^2_1 + 25X_1X_3 +12 X^2_2 -21 X_2X_3 +28 X^2_3$
Entrapment Efficiency (Y_2)	$61.85 + 9.37X_1 + 2.15 X_2 +1.89 X_3 +0.54X^2_1 - 2.74X_1X_3 -09.55 X^2_2 -2.50 X_2X_3 -3.44 X^2_3$
% Cumulative drug released (Y_3)	$76.67 +14.52 X_1 + 6.77 X_2 -14.39 X_3 +1.82X^2_1 -13.91X_1X_3 +4.14 X^2_2 -24.15 X_2X_3 +3.53 X^2_3$

Where Y1, Y2 and Y3 are the predicted response and X1, X2 and X3 are the coded values of the test variables in respective concentrations.

Particle size

For Niosomes, particle size is a crucial factor. A higher interfacial surface area is available for medication absorption with smaller particle sizes. Furthermore, a smaller particle size can allow for a quicker release rate. As demonstrated in Table 2, the nanoparticles' particle sizes were discovered to fall between 135-223 nm. The quadratic model created showed that the particle size is significantly influenced by the amounts of cholesterol, brij 72, and diethyl phoshate. Theoretical (predicted) values and observed values have a largely satisfactory level of agreement. The mathematical model generated for Particle size (Y1) was found to be significant with F-value of 0.0182 implies the model is significant. There is only a 0.02% chance that a "Model F-Value" this large could occur due to noise. The independent variables

A, B, C and the quadratic term of AB, BC, A2 and B2 have significant effects on the droplet size, since the P values less than 0.0500 represent the significant model terms as shown in Table 5. The "Lack of Fit F-value" of 0.0281 implies the Lack of Fit is significant relative to the pure error. There is a 01.16% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit is good, we want the model to fit. Results of the equation indicate that the effect of C is more significant than A and B. The factorial equation for droplet size showed a good correlation coefficient (0.9996). The influence of the main and interactive effects of independent variables on the particle size was further elucidated using the perturbation, contour and 3D response surface plots.

Table 5: ANOVA of the quadratic model for the response particle size (Y1)

Source of variations	Sum of squares	Degree of freedom	Mean squares	F-value	p-value Prob > F	R^2
Model	2765.27	6	460.87	0.0182	< 0.05	0.9996
A-Cholesterol	88.12	1	88.12	0.0293	< 0.05	
B-Brij 72	751.06	1	751.06	0.0345	< 0.05	
C-Diacetyl phosphate	12.53	1	12.53	0.0256	< 0.05	
AB	2245.12	1	2245.12	0.0385	< 0.05	
AC	1993.45	1	1993.45	0.0147	< 0.05	
AB	1.16	1	1.16	0.0340	< 0.05	
Residual	3436.23	6	572.66			
Lack of Fit	3874.62	6	645.89	0.0281	< 0.05	

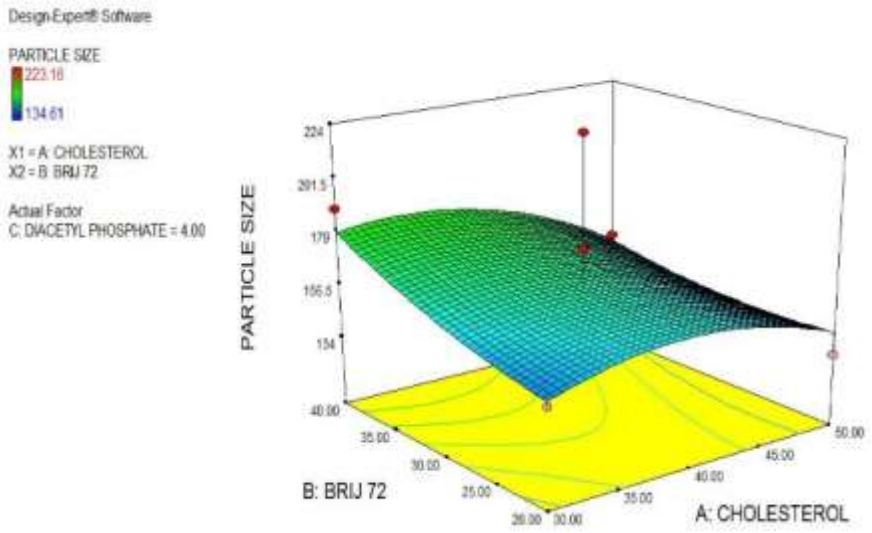

Figure 9 Response 3D surface plot showing the influence of amount of Cholesterol and amount of Brij 72 on particle size fixed level of C

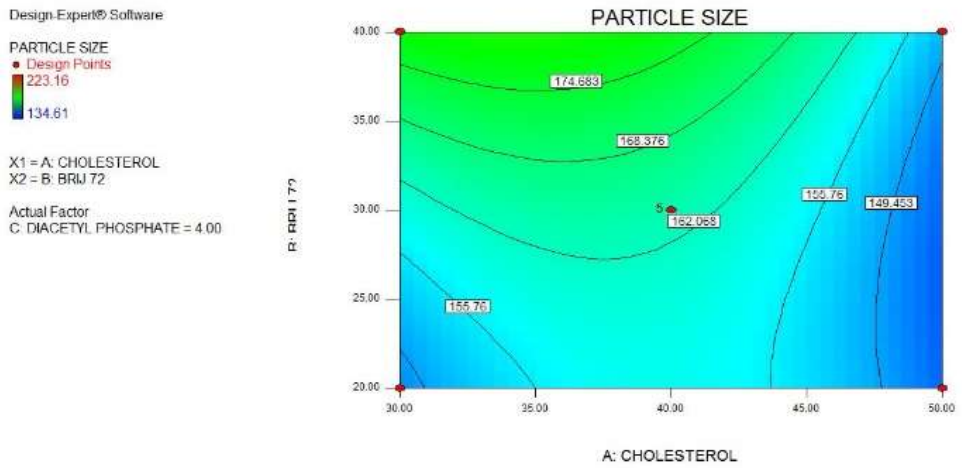

Figure 10 Contour plot showing the influence of amount of Cholesterol and amount of Brij 72 on particle size fixed level of C

Entrapment Efficiency (%)

According to the data in the table, the Niosomes' Entrapment Efficiency (percent) ranges from 73.2 to 86.4 percent. The quadratic model created showed that Brij 72 and cholesterol significantly affect the Entrapment Efficiency (percent). As can be seen, there was a respectable level of agreement between the theoretical (predicted) values and the observed ones. With an F-value of 0.0137, the mathematical model created for Entrapment Efficiency (percent) (Y2) was determined to be significant. The mathematical model generated for Entrapment Efficiency (%) (Y2) was found to be significant with F-value of 0.0137 implies the model is significant. There is only a 0.89% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B and C are significant model terms. as shown in Table 6. The "Lack of Fit F-value" of 0.0165 implies the Lack of Fit is significant relative to the pure error. There is a 1.73 % chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit is good,we want the model to fit. Results of the equation indicate that the effect of C is more significant than A and B. The factorial equation for Entrapment Efficiency (%) showed a good correlation coefficient (0.9992). The influence of the main and interactive effects of independent variables on the Entrapment Efficiency (%) was further elucidated using the perturbation, contour and 3D response surface plots.

From the results in Table 3, It was observed that the entrapment efficiency of niosomes composed of span 60 were superior as compared to those prepared from span 20. The formulation containing span 80 showed the lowest entrapment efficiency. This can be due to, the hydration temperature used to make niosomes should usually be above the gel to liquid phase transition temperature of the system that results in niosomes that are less leaky and have high entrapment efficiency. Span 60 has highest phase transition temperature (50 C) as compared to span 20 (16 C) and span 80 (

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12 C) and hence high entrapment efficiency and the length of alkyl chain of surfactant has a prominent effect on permeability of prepared niosomes. As the length of surfactant increases,

entrapment efficiency also increases. Span 60 has a longer saturated alkyl chain (C18) compared to span 20 (C12), so it produces niosomes with higher entrapment efficiency. Span 60 and span 80 have the same head group but span 80 has an unsaturated alkyl chain which results in enhanced permeability and decreased entrapment.

Table 6: ANOVA of the quadratic model for the response Entrapment Efficiency (%) (Y2)

Source of variations	Sum of squares	Degree of freedom	Mean squares	F-value	p-value Prob > F	R^2
Model	1287.88	6	214.56	0.0137	< 0.05	0.9992
A-Cholesterol	19.80	1	19.80	0.0187	< 0.05	
B-Brij 72	62.31	1	62.31	0.0338	< 0.05	
C-Diacetyl phosphate	35.12	1	35.12	0.0156	< 0.05	
AB	160.84	1	160.84	0.0256	< 0.05	
AC	180.10	1	180.10	0.0347	< 0.05	
AB	311.80	1	311.80	0.0240	< 0.05	
Residual	447.24	9	49.63			
Lack of Fit	253.17	6	42.16	0.0165	< 0.05	

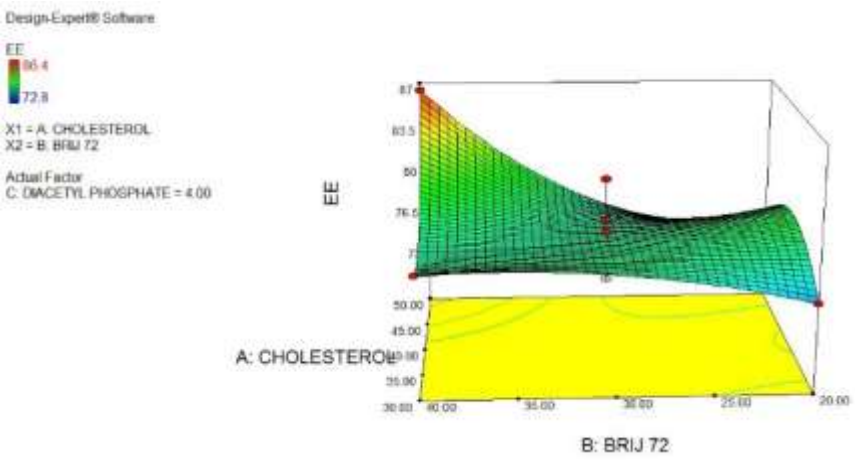

Figure 11 Response 3D surface plot showing the influence of Cholesterol and Brij 72 on Entrapment Efficiency (%) fixed level of C

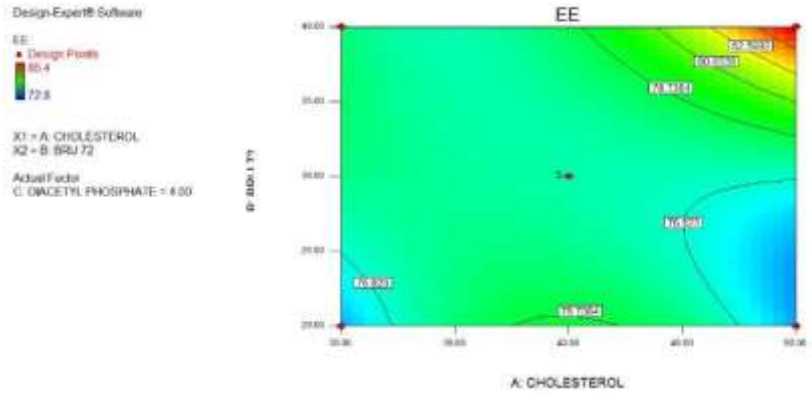

Figure 12 Contour plot showing the influence of Cholesterol and Brij 72 on Entrapment Efficiency (%) fixed level of C

Cumulative percent drug released

The cumulative percent drug release over the course of 24 hours from niosomes was discovered to be between 78.43% and 98.91 %. The quadratic model created showed that the particle size is significantly influenced by cholesterol, Brij 72, and diethyl phosphate. Theoretical (predicted) values and observed values have a largely satisfactory level of agreement. The mathematical model generated for percent drug released in 24 hrs (Y3) was found to be significant with F-value of 0.0274 implies the model is significant. There is only a 0.05% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, AB, AC, BC, A2, B2 are significant model terms as shown in Table 7. Values greater than 0.1 indicate the model terms are not significant. The "Lack of Fit F-value" of 0.0323 implies the Lack of Fit is significant relative to the pure error. There is a 01.25% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit is good, we want the model to fit.
Results of the equation indicate that the effect of C is more significant than A and B. The factorial equation for percent drug release showed a good correlation coefficient (0.9997). The influence of the main and interactive effects of independent variables on the particle size was further elucidated using the perturbation, contour and 3D response surface plots.

Table 7: ANOVA of the quadratic model for the response Cumulative percent drug released (Y3)

Source of variations	Sum of squares	Degree of freedom	Mean squares	F-value	p-value Prob > F	R^2
Model	1439.07	6	239.83	0.0274	< 0.05	0.9997
A-Cholesterol	30.77	1	30.77	0.0167	< 0.05	
B-Brij 72	11.45	1	11.45	0.0298	< 0.05	
C-Diacetyl phosphate	89.17	1	89.17	0.0395	< 0.05	
AB	41.38	1	41.38	0.0143	< 0.05	
AC	78.57	1	78.57	0.0139	< 0.05	
AB	63.70	1	63.70	0.0261	< 0.05	

Residual	902.30	9	100.22			
Lack of Fit	667.01	6	111.16	0.0323	< 0.05	

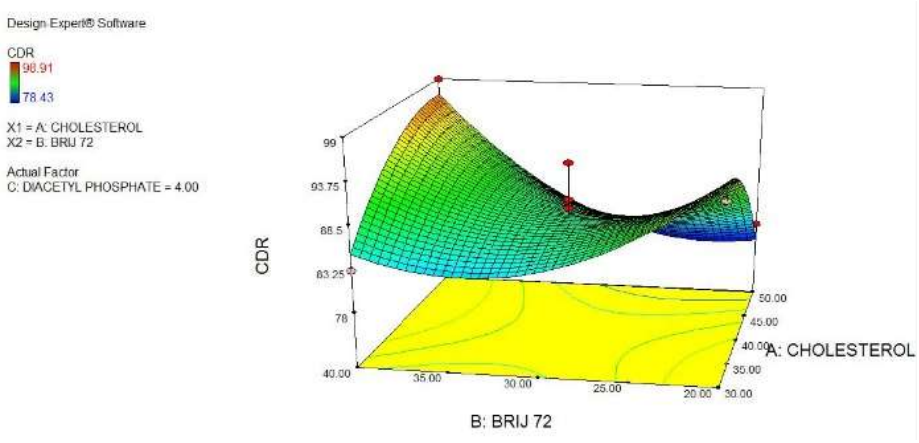

Figure 13 Response 3D surface plot showing the influence of Cholesterol and Brij 72 on Cumulative % Drug Released fixed level of C

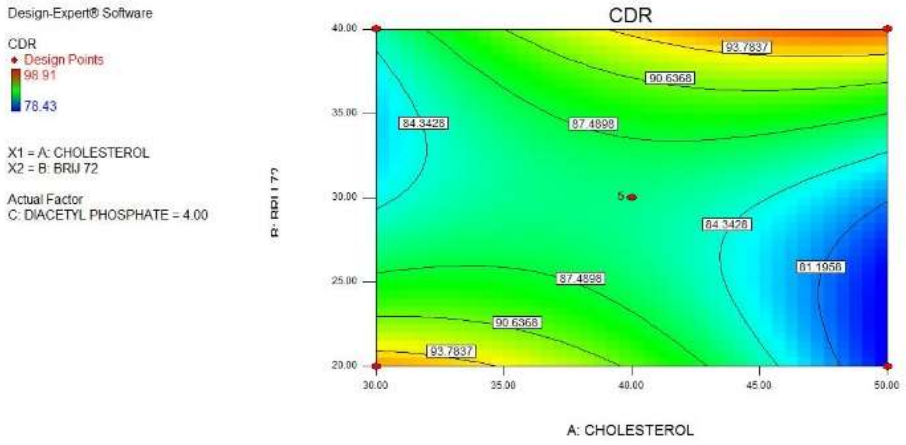

Figure 14 Contour plot showing the influence of Cholesterol and Brij 72 on Cumulative % Drug Released fixed level of C

Kinetic Data / Model fitting

Each kinetic's coefficient of correlation was determined and compared. The optimized Valacyclovir niosome formulation, known as VF13, has an in vitro drug release profile that fits a zero order model. The data was then handled in accordance with Korsmeyer's equation.

CONCLUSION

In this study, an effort was made to create and assess a niosomal drug delivery system for a particular antiviral medicine, such as Valacyclovir, in order to assure acceptable drug release, prolong the duration of action there by improve bioavailability and decrease the dosing frequency. Total 17 (VF1-VF17) formulations were made using a three-factor, three-level Box-Behnken design. Thin film hydration was used to make niosomal formulations. Diethyl

phosphate, cholesterol, and a surfactant were included in the formulation to serve as a negative charge inducer. This improves drug delivery effectiveness and prolongs the stability of the niosomal formulation. Various evaluation tests such as Zeta potential, Drug Content, Polydispersity Index, Entrapment Efficiency and Cumulative percent of drug release were performed to optimize the formulation. The Cumulative percent drug release of all prepared formulation shown (VF1-VF17) between 78.43 - 98.91%. Among the all formulations VF13 had shown 98.91% of drug release at 24 hrs. zeta potential parameter explain the stability of niosomes.

The particle sizes were discovered to fall between 135-223 nm. The quadratic model created showed that the particle size is significantly influenced by the amounts of cholesterol, brij 72, and diethyl phosphate. It is useful regarding the stability of the niosomes, less the particle size more the chance of stability of formulation. Percent Entrapment Efficiency (percent) ranges from 73.2 to 86.4 percent. The % Entrapment Efficiency is an important parameter as it denotes that whether all the formulations have the ability to entrap the drug in vesicles or not. The quadratic model created showed that Brij 72 and cholesterol significantly affect the Entrapment Efficiency (percent) and the cumulative percent drug release over the course of 24 hours from niosomes was discovered to be between 78.88 and 98.91 percent. The quadratic model created showed that the particle size is significantly influenced by cholesterol, Brij 72, and diethyl phosphate[14].

Our findings showed that all formulations had PDI values varying between 0.3 and 0.4, indicating that there was variance in the particle size distribution. The formulation VF13 was chosen as the optimum formulation out of all the formulations VF1 to VF17 since it demonstrated greatest release and other features. The formulation VF13 was determined from the results above to be the optimal formulation for the valacyclovir niosome that conformed with all the parameters. The optimized Valacyclovir niosome formulation, known as VF13, has an in vitro drug release profile that fits a zero order model. The data was then handled in accordance with Korsmeyer's equation.

In conclusion, valacyclovir loaded niosomes can substantially improve drug permeation; thereby offering clear-cut advantages over conventional dosage forms. Before findings of this investigation can be commercially realized, the detailed clinical investigations with special emphasis on efficacy and side effects are to be accomplished for success in market.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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