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Research Article

Formulation and Evaluation of Niosomal Gel Containing Acyclovir by Using Design of Experiments

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ABSTRACT

The current study aims to design, optimize, and assess the niosomal gel containing acyclovir for topical application. Compatibility analysis was carried out to assess potential interactions using techniques such as FTIR spectroscopy and DSC thermal analysis. The niosomal suspension was created, optimized, and assessed using a 3^2 - factorial design. The overlay plot indicates that the F3 formulation was optimized with a 300:20 tween 80 and cholesterol ratio. The improved niosomal solution has been introduced into a gel made of carbopol and thoroughly analyzed for pH, viscosity, spreadability, and *in-vitro* drug release characteristics. The study results demonstrated that the niosomal gel formulation exhibited a sustained release of acyclovir over a 24-hour period. The drug release data was assessed, and the findings unveiled that the medication release from the gel formulation complies with the Korsmeyer Peppas model. The optimized formulation's stability was demonstrated at various temperatures. The present investigation suggested that the niosome gel formulation (F3) could be a promising vesicular approach for effectively administering acyclovir *via* topical application.

Introduction

In recent years, topical skin therapy has drawn an enormous amount of interest in order to treat cutaneous disorders. However, topical administration of dermatological formulations provides improved patient compliance, less systemic absorption, and increased bioavailability.[1] Topical preparations are formulations that are smeared, rubbed, sprayed, or infused directly onto an exterior body surface. The topical technique of administration has been used to either induce systemic pharmacological effects or a local effect for treating skin disorders. In the primary category of semi-solid formulations, transparent gels have been used more often in dermatological and therapeutic applications. Some of the advantageous characteristics of gels intended for use in dermatology are as follows: they are soothing, non-staining, thixotropic, greaseless, easily transmissible, and removable. They can also be water-soluble.[2]

Niosomes are lipid-based vesicular membranes or aqueous layers that may enclose hydrophilic and lipophilic medicines, which are non-ionic surfactant vesicles. Niosomes, unlike liposomes, are more persistent vesicles that may be either unilamellar or multilamellar in nature. After combining cholesterol with a non-ionic surfactant, niosomes are created and then hydrated in an aqueous medium. Furthermore, cholesterol stiffens the bilayer and prevents niosomes from leaching.[3] Drug delivery *via* niosomes is a potential approach. Niosome encapsulation of drugs can reduce drug inactivation, reduce medication breakdown after administration, enhance drug bioavailability and targeting to the diseased area, and avoid unwanted side effects. Surfactants can break through the mucous barrier and act as penetration enhancers as well.[4] Topical application of niosome gel is preferable over conventional semi-solid formulations because it maintains superior rheological behavior, a more

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prolonged half-life, and a greater amount of medication in the skin. Niosomes have improved drug permeability through the skin. Noisome improves the stratum corneum properties by reducing transepidermal water loss and improving skin condition by reloading lost skin lipids. Niosomes can protect drugs from biological enzymes and acids, increasing their stability.[1]

Acyclovir is currently available for use in preventing and treating viral infections known as the varicella-zoster (VZV) and the Herps simplex viruses (HSV). Acyclovir possesses a 15 to 30% bioavailability when taken orally and a brief biological half-life of 2.5 hours. Traditional dosage forms of acyclovir require multiple daily doses. Consequently, the medication can be encapsulated in niosomes, a novel delivery technology, to sustain therapeutic concentration in the circulatory system over a longer duration of time, increasing the drug's bioavailability.[5]

Acyclovir 5% cream is frequently prescribed alongside oral tablets to effectively treat herpes labialis. The therapeutic effectiveness of these formulations is hindered due to limited drug penetration from topically applied substances over the lipid barrier, and oral dosage formulations typically have low bioavailability, typically falling within the range of 15 to 20%. The BCS class III drug acyclovir has restricted permeability. Improving acyclovir's ability to penetrate through the lipid membrane will improve the therapeutic result.[6]

Acyclovir is categorized as BCS class 3, characterized by its high solubility yet low permeability. As niosomes are gaining traction in topical drug delivery due to their remarkable characteristics and the effects they impart in formulations, including augmented drug permeability, acting as a reservoir for sustained drug release locally, and a rate-limiting membrane for modulation of systemic drug absorption *via* the skin. Therefore, endeavors were made to incorporate acyclovir into niosomes, alongside evaluating particle size and medication release characteristics.

Material And Methods

Acyclovir **(**VRL Pharma Tech, Vijayawada), Tween® 80 (Loba Chemie Pvt. Ltd. Mumbai), cholesterol (Loba Chemie Pvt. Ltd. Mumbai), chloroform (Thermo Fisher Scientific Pvt. Ltd. Mumbai), carbopol 934 (Loba Chemie Pvt. Ltd. Mumbai), glycerol (Thermo Fisher Scientific Pvt. Ltd. Mumbai), methylparaben (Alphamed Formulations, Hyderabad), propylparaben (Thermo Fisher Scientific PVT. Ltd. Mumbai), triethanolamine (Thermo Fisher Scientific Pvt. Ltd. Mumbai), FTIR (Alpha Bruker), DSC (TA Instruments), UV-visible spectrophotometer (Lab India Double Beam UV 3092), Rotary evaporator (Lab India), Particle size analyzer (Malvern Panalytical (HORIDA SZ-1000)), Brook field viscometer (Brookfield engineering laboratories), Franz diffusion cell (Orchid Scientifics™).

Reagent Preparation

Preparation of a phosphate buffer solution at pH 7.4

Developing a phosphate buffer solution with a pH of 7.4 necessitated precisely measuring 50 mL of potassium dihydrogen orthophosphate (0.2 M) and a 200 mL volumetric flask was filled with 39.1 mL of sodium hydroxide (0.2 M). By adding distilled water to the mixture, the amount of solution was raised to 200 mL, and the pH was balanced to 7.4 by adding 0.2N NaOH.

Creating an acyclovir standard solution

A 10 mg dose of the medication was dispersed in a 10 mL solution of phosphate buffer with a pH of 7.4 to create a standard stock solution (1000 µg). Take 1-mL of the standard stock solution and dilute it using a pH 7.4 buffer (100 µg). Further dilutions were conducted to acquire concentrations of 2, 4, 6, and 10 using pH 7.4 phosphate buffer to measure absorbance at λ_{max} 252 nm.

Preparation of acyclovir niosomal suspension with DoE

Niosomes were generated employing the conventional thin-film hydration process. To optimize a formulation with three levels and two factors, a 3^2-level complete factorial design was chosen. Design of acyclovir niosomes using independent variables Tween 80 (X1) and cholesterol (X2). The concentration levels are 100, 200, 300 and 20, 40, and 60, respectively and the dependent variables were particle size (Y1), zeta potential (Y2) and *in-vitro* drug diffusion (Y3) utilizing a design expert 11 software nine different combinations were formulated and assessed.

Niosomes were prepared by utilizing a lipid mixture comprising cholesterol and surfactant (tween 80) at various predetermined ratios, as shown in Table 1. The drug (Acyclovir) 100 mg, tween 80, and cholesterol were all mixed together in 10 mL of chloroform. A rotary flash evaporator was employed to vaporize the liquid at 45°C until a thin lipid film developed at 70 rpm. The created film had been moisturized with 20 mL of distilled water and maintained hydration for half an hour. The hydrated niosomes were homogenized for 30 minutes with a high-

Table 1: Formulation table for niosomes

S. No.	*Ingredients*	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*F7*	*F8*	*F9*
1.	Acyclovir (mg)	100	100	100	100	100	100	100	100	100
2.	Tween 80 (mg)	100	200	300	100	200	300	100	200	300
3.	Cholesterol (mg)	20	20	20	40	40	40	60	60	60
4.	Chloroform (mL)	10	10	10	10	10	10	10	10	10

speed homogenizer, yielding a niosomal slurry containing both unbound and bound drugs of varying sizes.[7]

Evaluation of Acyclovir Niosomes

Measurement of particle dimensions and zeta potential

The particle dimension and zeta potential of the niosomal solution have been assessed by employing a particle size analyzer (HORIBA SZ-100). Upon introducing 1-mL of the sample containing niosomes into the empty sampling chamber, a software system has been used to determine the average size of the particles.[8] Other measures, including vesicle charge and average zeta potential, were measured directly. The zeta potential was studied in order to better understand vesicle stability and colloidal characteristics.[9]

Entrapment efficiency

The drug's entrapment efficiency was assessed using centrifugation. Niosomal suspension samples were centrifuged using a laboratory centrifuge (Remi R4C). A UV-visible spectrophotometer set to 252 nm was employed to quantify the amount of medication in the clear supernatant collected after centrifugation. The quantity of acyclovir contained in niosomes was ascertained.[10]

$$\%\ \text{Drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid}) \times 100}{\text{Total drug}}$$

Percentage yield

To calculate the percentage yield of niosomal suspension and improved niosomal gel, divide the actual weight of the formulations by the formula weight and multiply by 100.[11]

$$\%\text{practical yield} = \frac{\text{Practical yield} \times 100}{\text{Total theoretical yield}}$$

In-vitro drug release studies

The membrane diffusion approach was employed to assess acyclovir dissolution from a niosomal formulation at different ratios. A dialysis membrane (HI Media, molecular weight 5000) is inserted, separating the donor and receptor compartments. Acyclovir niosomal suspension was introduced to the donor compartment, followed by pH 7.4 phosphate buffer in the receptor compartment. The diffusion cells have been maintained at 37 ± 0.5°C and agitated at 300 rpm during the experiment. To uphold sink conditions, 5 mL of specimens were collected at consistent time points and supplemented with an additional 5 mL of freshly prepared buffer. The collected samples have been evaluated using an ultraviolet spectrophotometer at 252 nm.[12]

Preparation of niosomal gel

Carbopol gel was created by mixing 1-g of carbopol in a 7:3 water-glycerol combination. The mixture was agitated until thickened using a mechanical stirrer. Other components, such as methylparaben and propylparaben, were then incorporated while stirring continually. The addition of triethanolamine neutralizes and viscosities the dispersion. An optimized niosomal suspension containing 100 mg of acyclovir was incorporated into the prepared carbopol 934 base gel.

Evaluation of Niosomal Gel

pH

The niosomal gel formulation's pH was assessed utilizing a digital pH meter. After dissolving 1 g of gel in 100 mL of pure water, let it stand for a couple of hours. For every formulation, the pH was evaluated three times, and then the average value was ascertained. [13]

Viscosity

The viscosity was assessed using a Brookfield viscometer. Viscosity has been determined at an ambient temperature (25–27°C) employing a Brookfield viscometer. [14]

Spreadability

A 0.5 g sample of the optimal gel was sandwiched between two glass slides and allowed to set in about 5 minutes, after which additional spreading was not expected. Spread circle diameters were measured in centimeters and used as standards for spreadability. [2]

Drug content

By dissolving precisely weighed 1 g of gel into a phosphate buffer with a pH level of 7.4, the medication concentration in the gel could be determined. After a suitable dilution, absorbance at 252 nm was measured utilizing a UV spectrophotometer. The standard curve's slope has been utilized for assessing the amount of medication. The drug content was computed using the formula below. [15]

Drug Content = (Concentration × Dilution Factor × Volume taken) ×Conversion Factor

In-vitro diffusion studies

In-vitro diffusion investigations on the developed niosomal gel and pure drug have been conducted employing a Franz diffusion cell. A dialysis membrane has been utilized for distinguishing the donor and receptor compartments. Throughout the procedure, the temperature remains constant at 37°C. In contrast, stirring at 200 revolutions per minute to preserve the sink conditions. Pipette 5 mL of the solution from the receptor compartment at regular time intervals and replace it with 5 mL of freshly prepared phosphate buffer. Subsequently, the samples underwent testing at 252 nm employing an ultraviolet-visible spectrophotometer. [16]

Drug release kinetics

The acyclovir niosomal gel formulation's medication release profile, including zero order, first order, Higuchi, Koresmeyer Peppas, etc., is evaluated for release characteristics to assess the medication release process.

Appropriate statistical analysis is carried out using the appropriate release kinetics.[17]

Stability studies

Following the principles set forth by the International Council for Harmonization (ICH), a short-term stability study was conducted. The formulation was stored in an appropriate container at 4 to 8°C & 25 ± 2°C for 90 days. The medication concentration and *in-vitro* drug release of the samples were assessed.[18]

Results And Discussion

Preformulation Studies

Organoleptic properties

Acyclovir in its pristine form presented as a white, finely powdered substance, exuding a distinctive odor, and offering a taste ranging from acrid to alkaline.

Solubility profile

The solubility of acyclovir in various solvents was ascertained and is depicted in Table 2.

Melting point analysis

The capillary method has been employed for assessing the medication's melting temperature, yielding an estimated value of 256.5°C for acyclovir.

Calibration curve for acyclovir

The absorbance of acyclovir in a phosphate buffer with a pH of 7.4 obtained at various concentrations is shown in Fig. 1. The absorbance versus concentration graph for acyclovir showed a linear relationship between 2 to 10 µg/mL and R^2 value of 0.999.

FTIR studies

To assess the compatible nature of the medication and excipients, as well as the functional group, FTIR spectroscopy was performed on the pure form of acyclovir in Fig. 2. and the additives that are utilized in the formulation of niosomal gel in Fig. 3. As a result, FTIR investigations revealed that the medicine (acyclovir) had no interactions with the pharmaceutical excipients employed in the formulation (Table 3).

Differential Scanning Calorimetry

The thermographic images of acyclovir are displayed in Fig. 4. At 255.7°C, the DSC curve exhibited an endothermic peak. The DSC curve for the physical mixture exhibits

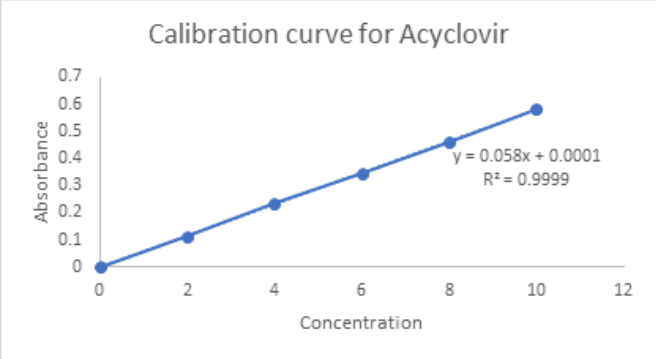

Fig. 1: calibration curve for acyclovir

Fig. 2: FTIR spectrum of acyclovir

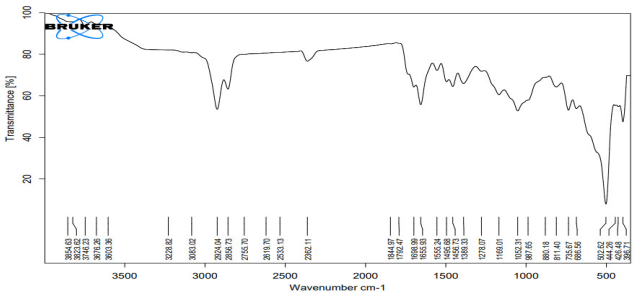

Fig. 3: FTIR spectrum of drug and excipients

Table 2: Solubility of acyclovir in various solvents

Name of the solvent	*solubility*
0.1N HCL	soluble
Water	Slightly soluble
Ethanol	Sparingly soluble
Methanol	Sparingly soluble
Chloroform	Soluble
pH 7.4 phosphate buffer	Soluble

Table 3: Reported and observed peaks of drug and physical mixture

Functional group	*Reported peak (cm^{-1})*	*Observed peak in drug (cm^{-1})*	*Observed peak in physical mixture (cm^{-1})*
-C=0	1740–1690	1699	1698
-COOH	3000–2500	2821	2856
-NH	3700–3500	3766	3676
-OH	3550–3200	3330	3228
C=C	1700–1500	1558	1555

Table 4: DoE generated 3^2 factorial design

Run	*Factor 1 a: tween 80*	*Factor 2 b: cholesterol*	*Response 1 (Particle size)*	*Response 2 (Zeta potential)*	*Response 3 (in-vitro diffusion studies)*
1	100	20	94.5 ± 0.45	-5.4 ± 0.21	66.27 ± 0.14
2	200	20	100.8 ± 0.92	-15.9 ± 0.34	71.68 ± 0.13
3	300	20	110.3 ± 1.18	-24.2 ± 0.42	83.94 ± 0.36
4	100	40	121.6 ± 1.27	-41.5 ± 0.69	68.84 ± 0.49
5	200	40	123.4 ± 1.29	-42.1 ± 0.71	75.64 ± 0.31
6	300	40	125.6 ± 1.31	-43.0 ± 0.74	88.48 ± 0.41
7	100	60	141.5 ± 1.52	-50.4 ± 0.81	81.47 ± 0.37
8	200	60	143.6 ± 1.55	-53.1 ± 1.48	79.46 ± 0.36
9	300	60	147.8 ± 1.59	-58.0 ± 1.21	93.66 ± 0.21

Mean ± SD, n=3

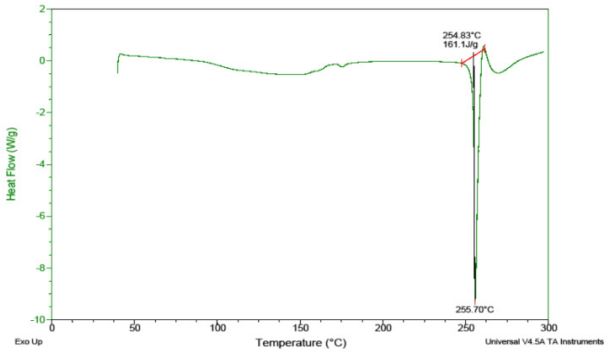

Fig. 4: Thermogram of acyclovir

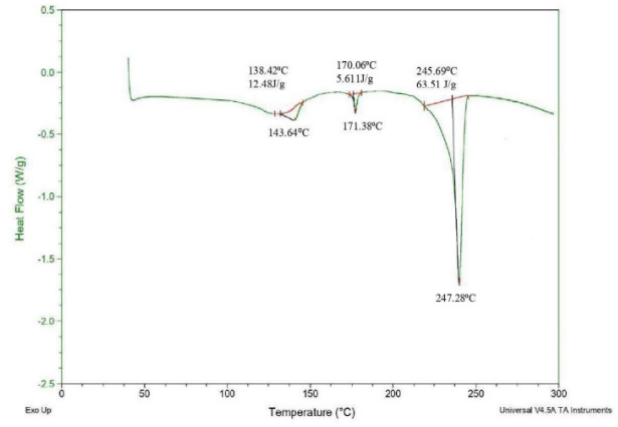

Fig. 5: Thermogram of physical mixture

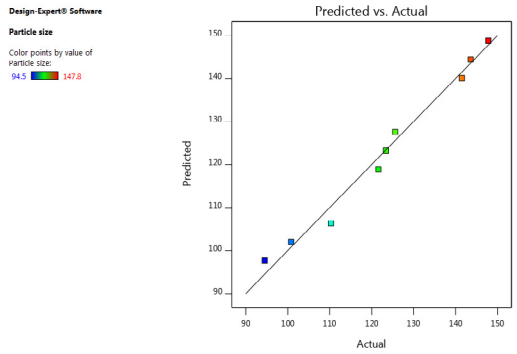

Fig. 6: Predicted *vs* actual plot for response1

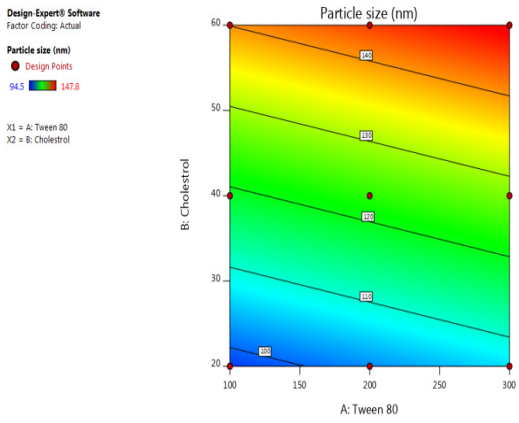

Fig. 7: Contour plot for response 1

an endothermic peak at 247.28ºC, the melting point of acyclovir in Fig. 5. A comparison of the endothermic peaks of the acyclovir DSC curve with the physical combination demonstrates the lack of interactions.

Design of Experiments

The generated niosomal gel was evaluated using various tests, including particle dimensions, zeta potential, and *in-vitro* diffusion investigations. These assessments were conducted using the Design Expert @ 11 software (Table 4).

Response 1 (particle size)

Particle size = +123.23+4.35*A+21.22*B

Analysis of variance (ANOVA) revealed the generated linear model was extremely significant, with a probability value of < 0.005. The R2 value was determined to be 0.9853. The

graph of observed particle size against predicted particle size (Fig. 6) exhibits an uninterrupted line. As a result, it is possible to conclude that the formula is highly predictive contour plot (Fig. 7). The three-dimensional diagram (Fig. 8) and regression coefficient values of components led to the conclusion that as the amount of Tween 80 and cholesterol increased, particle size increased. Due to the high hydrophilic nature of Tween 80 (HLB-15), it exhibits a larger particle size. Tween 80, on the other hand, is a non-ionic surfactant known for its ability to lower interfacial tension and maintain the stability of the niosome dispersion. Cholesterol is frequently included in niosome formulations to bolster membrane rigidity and stability. This characteristic typically decreases the fluidity of lipid bilayers, potentially resulting in larger particle sizes due to reduced deformability and increased rigidity of the membrane. In conclusion, cholesterol typically enlarges particle size by stiffening the niosome membrane. The combined effect of increasing both can lead to a nuanced impact on particle size.

Response 2 (Zeta potential)

- *Zeta potential = -37.07 -4.65*A-19.33*B*

The analysis of variance (ANOVA) confirmed that the generated linear model was very significant, as evidenced by an estimated probability of less than 0.005. The R2 value was identified as 0.9233. The graph of measured zeta potential against anticipated zeta potential (Fig. 9) indicates a line that is parallel. As a result, it is possible to conclude that the equation is highly predictive contour plot (Fig. 10). Based on the 3D plot (Fig. 11) as well as the regression coefficient values of variables, the threshold to distinguish between stable and unstable dispersions is typically +30 or -30 mV. In general, particles possessing zeta potentials larger than +30 or -30 mV are regarded as stable. The zeta potential values range from (-5.4–-58 mV). It was determined that by raising the concentrations of tween 80 and cholesterol, the negative values of zeta potential increase concurrently. Lipids and Tween 80 reduce electrostatic repulsion between particles and sterically stabilize niosomes by forming a protective coating around their surface, potentially attributing to this phenomenon.

Response 3 (In-vitro diffusion studies)

- *ID = +80.06+6.77*A+7.60*B*

The analysis of variance (ANOVA) demonstrated that the generated linear model was particularly significant, as proven by a probability value of less than 0.005. The R2 value was estimated to be 0.9228. The graph of observed ID vs. anticipated ID (Fig. 12) indicates a straight line.

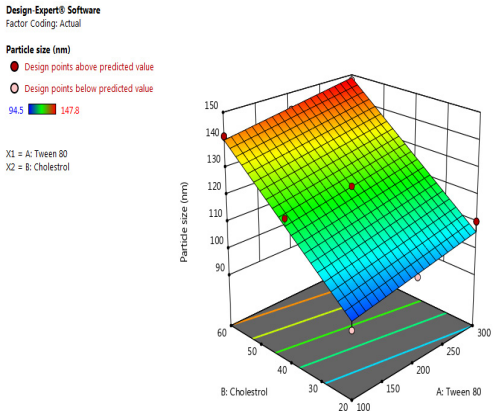

Fig. 8: 3D response surface plot for response 1

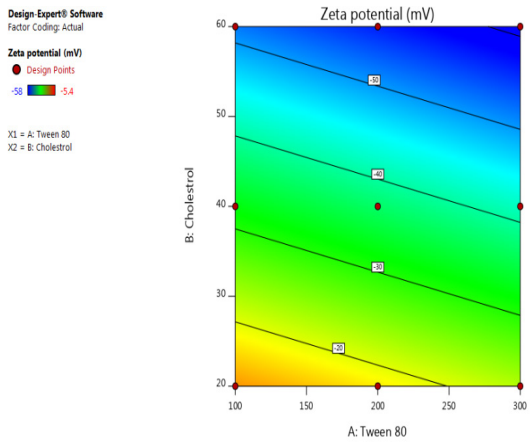

Fig. 10: Contour plot for response2

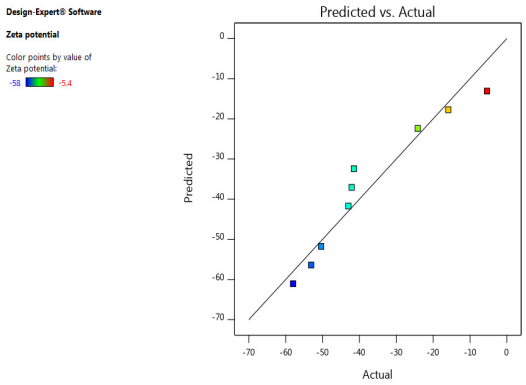

Fig. 9: Predicted vs Actual plot for response 2

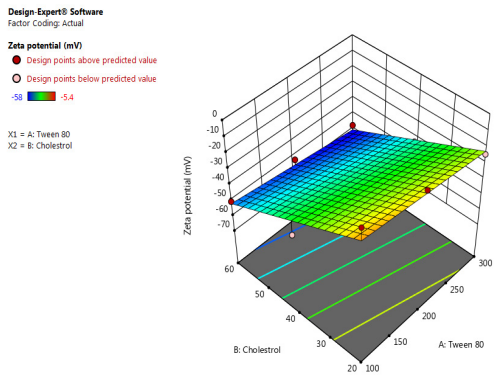

Fig. 11: 3D response surface plot for response 2

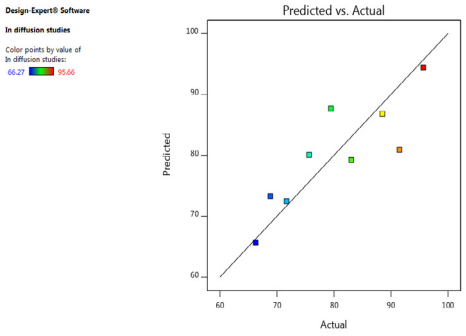

Fig. 12: Predicted vs Actual plot for response 3

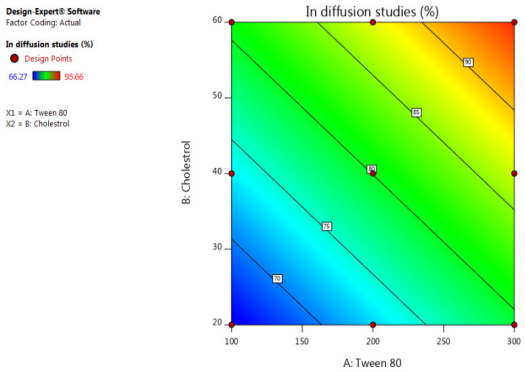

Fig. 13: Contour plot for response 3

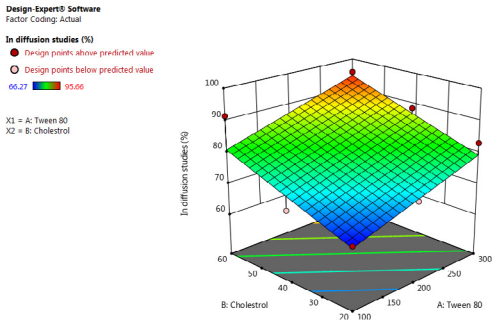

Fig. 14: 3D response surface plot for response 3

As a result, it is possible to conclude that the equation is significantly predictive contour plot (Fig. 13). Depending on the three-dimensional illustration (Fig. 14) and the values of regression coefficient variables, it was determined that a rise in the concentration of Tween 80, which has a hydrophilic-lipophilic balance of 15, had the maximum effect on drug release rate enhancement. Tween 80 can enhance the release of acyclovir by augmenting its solubility, stabilizing its formulation, altering its partitioning behavior, and facilitating its diffusion. Collectively, these effects significantly enhance the delivery and release of acyclovir for therapeutic purposes.

Design space

The contour plot for particle size, zeta potential, and *in-vitro* drug diffusion depicted a design space for the investigated concentrations of Tween 80 and cholesterol. The design space's limitations were defined by the central area of the contor plot, which was marked in yellow.

For validation purposes, the subsequent confirmatory checkpoint batches were produced within the acquired design space. The formulation for these confirmatory batches is illustrated in Figs 15 and 16.

Design expert 11.0 software is utilized to perform a factorial design analysis on *in-vitro* drug dissolution data. To generate and assess the improved niosomal gel, it was necessary to modify the acyclovir niosomal composition.

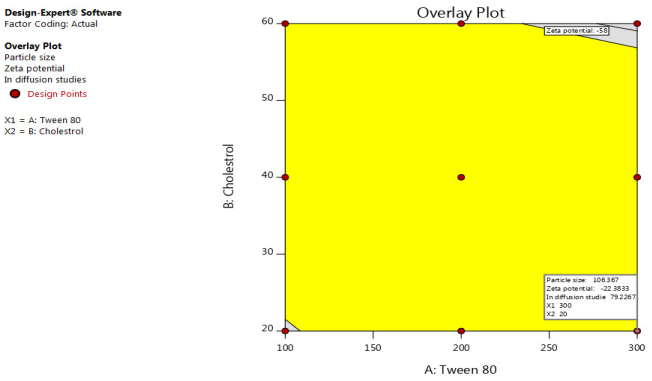

Fig. 16: Overlay plot

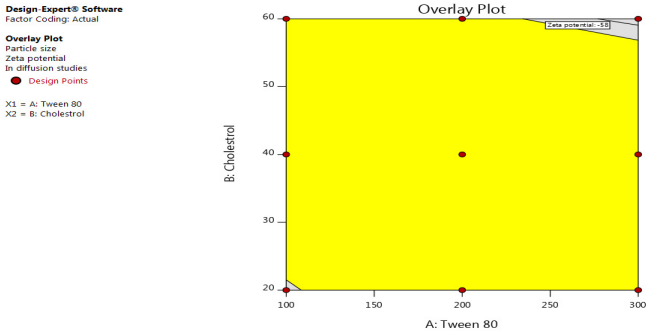

Fig. 15: Design space confirmatory trail

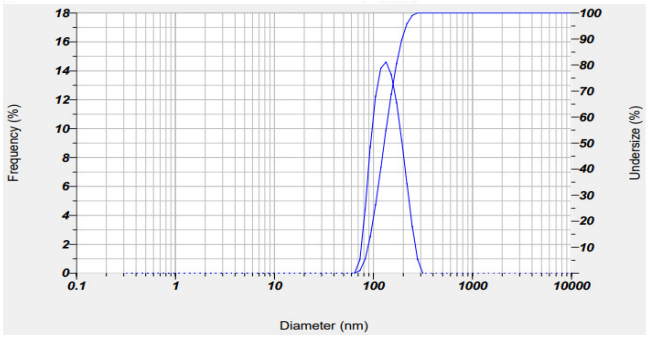

Fig. 17: Particle size of optimized formulation (F3)

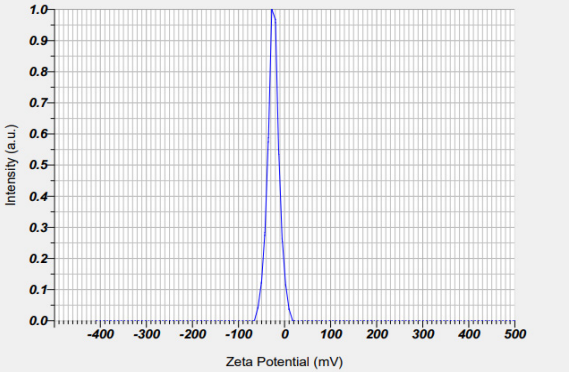

Fig. 18: Zeta potential of optimized formulation (F3)

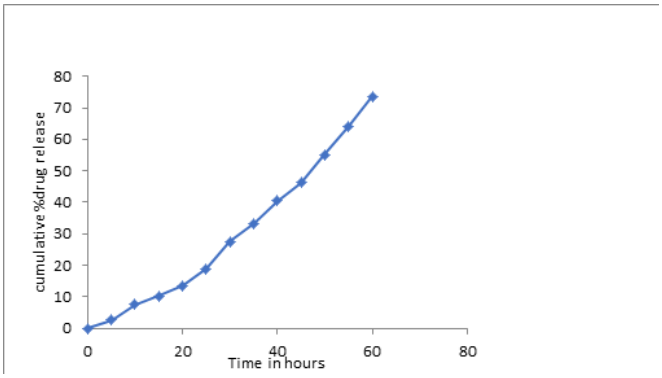

Fig. 19: *In-vitro* drug release of pure drug

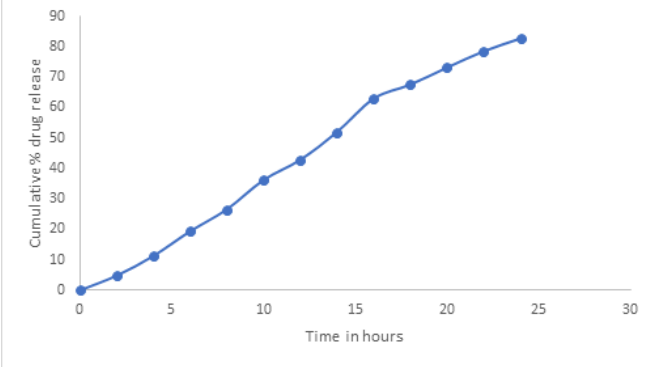

Fig. 20: *In-vitro* drug release of optimized formulation (F3)

According to the overlay plot of design expert software F3 formulation was an optimized formulation.

Particle size

The optimized formulation (F3) exhibited a particle size of 110.3 ± 1.18 and is shown in Fig. 17.

Zeta potential

The optimized formulation had a measured zeta potential of -24.2 ± 0.42 and is shown in Fig. 18.

%Drug entrapped

The entrapment efficiency of the optimized formulation was determined to be 87.53 ± 1.25%.

In-vitro diffusion studies

The optimal formulation F3, demonstrated an 83.94 ± 0.36%

Percentage yield

The yield of an optimized niosomal suspension was determined to be 90.51%.

pH

The ideal formulation's pH was obtained using a digital pH meter. The optimized formulation's pH proved to be 5.6, which falls within the acceptable pH range of the skin.

Viscosity

The improved niosomal gel's viscosity was assessed. The gel's viscosity was determined to be 3721 centipoise.

Spreadability

One of the most significant features of a semi-solid formulation is its spreadability. It was discovered that the improved formulation F3 possesses a spreadability of 7.4 g.cm/sec.

Drug content

The optimal formulation (F3) has a drug content of 80.56%. Since the percentage of drug did not vary noticeably, the drug content result indicated that the niosomal solution had been effectively disseminated throughout the gel.

In-vitro diffusion studies

In-vitro diffusion investigations for improved formulation (F3) and pure drug were carried out employing a Franz diffusion cell. The optimal formulation F3 achieved an 82.73% *in-vitro* drug release rate, while the pure drug demonstrated a 73.64% rate. These results are detailed in Table 5 and Figures 19 and 20.

Drug release kinetics

From the release kinetic parameters, the Korsmeyer peppas approach was the most suitable model for

Table 5: *In-vitro* drug release of pure drug and niosomal gel

Time (hours)	*%CDR of pure drug*	*%CDR of niosomal gel*
0	0	0
2	2.679	4.821
4	7.515	11.28
6	10.24	19.38
8	13.58	26.59
10	18.94	36.24
12	27.61	42.86
14	33.27	51.96
16	40.58	62.97
18	46.16	67.60
20	55.07	73.19
22	64.18	78.55
24	73.64	82.73

Table 6: Regression analysis of formulation 3

Formulation code	*Zero order* R^2	*First order* R^2	*Higuchi* R^2	*Korsmeyer Peppas*		*Hixson-Crowell* R^2	*Best fit model*
				R^2	*n value*		
F3	0.9928	0.9676	0.9823	0.995	1.163	0.973	Korsmeyer peppas

Table 7: Stability studies of acyclovir niosomal suspension

Time of storage (in days)	*Temperature of storage*			
	% Drug content at 4-8ºC	*In-vitro diffusion studies at 4-8ºC*	*% Drug content at 25 ± 2°C*	*In-vitro diffusion studies at 25 ± 2°C*
0	80.26	83.94	80.26	83.94
30	79.82	82.23	79.65	82.79
60	79.54	81.56	79.34	81.26
90	79.16	81.02	78.05	80.32

Table 8: Stability studies of acyclovir niosomal gel formulation (F3)

Time of storage (in days)	*Temperature of storage*			
	%Drug content at 4–8°C	*In-vitro diffusion studies at 4–8°C*	*%Drug content at 25 ± 2°C*	*In-vitro diffusion studies at 25 ± 2°C*
0	80.56	82.73	80.56	82.73
30	79.97	81.54	79.85	81.65
60	79.56	81.32	79.16	81.24

characterizing the behavior of the optimized composition (F3), with the highest regression coefficient value (R2). The drug releases according to Korsmeyer peppas kinetics, which resulted in n= 1.163, suggesting that the mechanism of drug release adheres to non-Fickian diffusion (Table 6).

Stability Studies of the Optimized Formulation

The acyclovir-improved niosomal formulation and niosomal gel (F3) underwent 90-day stability testing at room temperature (25 ± 2°C) as well as refrigerated temperatures (4–8°C) for short-term stability tests. Investigations of the medication content and *in-vitro* diffusion of acyclovir within the niosomes and niosomal gel were performed subsequent to the formulation and at 30-day intervals for an average of 90 days. These findings are presented in Tables 7 and 8. Medication leaking *via* vesicles was minimal, around 4 to 8°C, implying that the niosomal gel remained stable.

Conclusion

The research demonstrated the effective production of a niosomal gel incorporating acyclovir, which exhibited enhanced penetration capability, excellent permeability, and enhanced duration of effectiveness. Analysis *via* DSC thermographic images and FTIR spectra disclosed the absence of interactions between the medication and the excipients. Through the 3^2 -factorial design, the F3 formulation emerged as an optimized composition. Findings demonstrate that extended medication release *via* the niosomal gel implies a substantial reduction in dosage frequency, thus facilitating patient compliance. Administering medication as a niosomal gel formulation improves its ability to penetrate. The stability studies reveal that the composition maintains its stability at refrigerated temperatures with insignificant drug reduction.

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