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Original Article

Formulation and Evaluation of Acyclovir Loaded Aspasomal Gel for Effective Management of Herpes

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ABSTRACT

Objectives: The aim of the present study is to formulate and evaluate acyclovir loaded aspasomal topical gel by using Aloe vera gel base to enhance skin permeation and reduce the associated oral side effects of acyclovir. **Materials and Methods:** Acyclovir loaded aspasomes were formulated by using Ascorbyl Palmitate, Cholesterol and Chloroform: Methanol in 9:1 ratio by Thin Film Hydration Method. Phosphate Buffer Solution (PBS) pH 7.4 was used to hydrate the film obtained. The formulated aspasomes were further characterized for Drug Content, Vesicle Size, Poly Dispersibility Index (PDI) and % Entrapment Efficiency (EE). Optimized formulation of Acyclovir loaded Aspasomes was incorporated in Aloe gel base containing Carbopol 940 (1.5%). The formulated gels were characterized for pH, viscosity, spreadability, *in vitro* drug diffusion and *ex vivo* diffusion studies. **Results:** 3^2 Factorial Design (Design Expert Software) was used for the optimization study. The optimized Aspasomal Formulation (F8) showed % Entrapment Efficiency of 87.98±0.47%, Vesicle Size of 128.6±1.8 nm and Drug Content of 84.05±0.17%. The formulated aspasomal gel resulted to have a neutral pH value, with 1557±0.213 cps viscosity and 6.9±0.163 g.cm/sec spreadability. The *in vitro* diffusion studies (12 hr) and *ex vivo* diffusion studies (12 hr) revealed that aspasomal gel released the drug in the range of 0.715±0.11% to 87.48±0.42% and 63.56±0.36% respectively and that of marketed acyclovir gel released the drug in the range of 0.661±0.51% to 34.077±0.43% and 30.64±0.62% respectively, indicating the enhanced skin diffusion of aspasomal gel revealing that the formulated aspasomal gel is a better carrier than the marketed gel for transdermal application. **Conclusion:** Optimized Formulation (F8) showed better results in terms of vesicle size, % EE and drug release. Formulated aspasomal gel showed optimum results in terms of pH, viscosity, spreadability and percentage drug release. *In vitro* studies and *ex vivo* studies conclude that acyclovir loaded aspasomal gel revealed controlled drug release than the marketed gel (12 hr). The stability studies indicated no significant change in physical characteristics.

Keywords: Herpes, Acyclovir, Aspasomes, Herpes Simplex Virus.

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INTRODUCTION

Herpes Simplex Virus (HSV) is a double-stranded, enveloped, DNA virus. Herpes Simplex Virus type-1 (HSV-1) and type 2 (HSV-2) belongs to the family Herpesviridae, subfamily Alpha Herpesviridae. Following initial infection, the herpes virus becomes latent in the sensory neural ganglia (the trigeminal ganglion in HSV-1 infection and the sacral ganglion in HSV-2 infection).[1]

The most used antiviral drug for treating HSV infections is Acyclovir. Famciclovir, Valacyclovir, Penciclovir, Foscarnet, etc. are some of the other currently used oral medicines. They exhibit major drawbacks such as, headache, nausea, diarrhea and some severe adverse effects like hepatotoxicity and Steven Johnsons Syndrome.[2] The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme Thymidine Kinase (TK) encoded by Herpes Simplex Virus (HSV) and Varicella Zoster Virus (VZV). Acyclovir (ACV) has low oral bioavailability of 10-20%. ACV being the most potent and first line drug for HSV, because of its affinity for HSV-thymidine kinase, which competitively reduces viral DNA replication. Viral thymidine kinase first changes Acyclovir into Acyclovir-monophosphate after it enters infected cells. It is then further phosphorylated into acyclovir triphosphate by host cell kinases. This active form spares host cell activities while interfering with the production of viral DNA, hence preventing viral replication.[2] It is administered by the oral, topical and intravenous route in herpes infection.[3] On oral administration, ACV exhibits general side effects which include anaphylaxis, angioedema, fever, headache, pain, peripheral edema, gastrointestinal disturbances like diarrhea and

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nausea.[4] Because of its limited permeability and high solubility, acyclovir is classified as BCS class III. Therefore, a novel drug formulation is preferred for its effective administration.

Aspasomes are ascorbyl palmitate vesicle with biological activity. Ascorbyl Palmitate (ASP) is explored as bilayer vesicle forming material. It forms the vesicles (Aspasomes) in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). ASP is ascorbic acid esters with amphiphilic nature and has surface-active properties. It is capable to suppress pigmentation of the skin and decomposition of melanin. It also improves elasticity of the skin by promoting the formation of collagen. ASP is more stable than ascorbic acid. Its lipophilic character is beneficial for its skin penetration.[5] Thus; aspasomes can encapsulate acyclovir and increase its permeability, allowing for effective absorption. Their tiny size promotes cellular absorption, enhancing acyclovir's anti-Herpes activity while bypassing BCS class III limitations.

Aloe Vera, a perennial succulent plant that can withstand droughts, is well known for its healing properties.[6] Aloe Vera has been shown to have a range of positive effects, such as immunomodulatory, burn and wound healing, hypoglycaemia, anticancer, gastro-protective, antifungal and anti-inflammatory characteristics.[7]

Previous transdermal acyclovir nano-formulations like Niosomes,[8] Liposomes[9] have been formulated. In niosomes, the method of formulation is time consuming and requires special equipments. Incomplete hydration of surfactants may cause the sediment to accumulate. The entrapped drug may fuse or leak during storage.[10] In case of liposomes, the method of preparation is time-consuming, difficult to formulate and scale-up is a difficult task as sterilization is required in some cases. Water-soluble drugs exhibit low entrapment efficiency and the removal of organic solvent which is used in large quantity can be a tedious step.[11]

Aspasomes on the other hand, offer many advantages over previously formulated Acyclovir nano-formulations as, topical therapy can be accomplished through sustained, targeted and controlled drug release. Increased therapeutic concentrations locally, encapsulation of hydrophobic, lipophilic and amphiphilic drugs is easily possible. Aspasomes have skin whitening effect by reducing skin pigmentation; promote collagen formation, which aids in skin elasticity.[7]

Thus, the aim of the present study was to formulate and evaluate acyclovir loaded aspasomal topical gel by using aloe vera gel base which enhances skin permeation of drug and also reduces the associated oral side effects of acyclovir such as first pass metabolism, upset stomach, nausea, vomiting, diarrhea, dizziness, tiredness and seizures.

MATERIALS AND METHODS

Acyclovir was provided as gift sample from NEON Pharmaceuticals, Mumbai. Cholesterol was obtained from Hi-Media Ltd., Mumbai and Ascorbyl Palmitate was obtained from Sigma Aldrich Ltd., Mumbai.

Preformulation Studies

Identification test

Standard Calibration Curve for Acyclovir in PBS pH 7.4

Acyclovir dilutions of concentrations from 2-10 µg/mL were found to be in Beer's range. To prepare the stock solution of acyclovir, 10 mg of ACY was added to 100 mL volumetric flask, slight quantity of ethanol (5-7 mL) was used to dissolve the drug and the volume was made up to the mark with PBS pH 7.4, to achieve a concentration of 100 µg/mL. Aliquots were taken (0.2, 0.4, 0.6, 0.8, 1 mL) and the volume was made-up to 10 mL with PBS pH 7.4 to get the concentration 2 to 10 µg/mL and analyzed by UV spectrophotometer at a wavelength of 256nm (Table 1).[12-17]

IR Spectroscopy

Fourier Transform-Infrared (FT-IR) spectrum was compared to a typical FT-IR spectrum of pure drug as a reference. IR spectroscopy was performed to identify the compatibility of the drug with the excipients. Hence, FT-IR analysis of Acyclovir and physical mixture i.e., Acyclovir, Cholesterol, Ascorbyl Palmitate and Carbopol 940 was performed. All the spectra were scanned at a resolution ranging from 5 cm^{-1} to 5000 cm^{-1}.[18]

Formulation of Aspasomes

Acyclovir loaded aspasomes were prepared by using Thin Film Hydration Method.[19-22] Briefly, weighed quantity of Acyclovir, Ascorbyl Palmitate and Cholesterol were dissolved in Chloroform: Methanol solution in 9:1 ratio in a beaker and covered with aluminum foil. Then this solution was transferred in the round bottom flask (Rota evaporator's RBF) and sonicated for 2 min (Bath Sonicator). Further, the RBF was attached at the receptor compartment of the Rota evaporator. The temperature of water bath was maintained at 37°C to 40°C and the rpm was set between 30 to 40. After the vacuum pressure came down to 200 Pa, the whole solvent was evaporated and a Thin Film was observed inside the RBF. Further, the RBF was kept into a desiccator and after 24 hr, the film was then hydrated with PBS~pH 7.4 buffer (Table 2).

Evaluation of Aspasomes

Determination of Drug Content in Acyclovir Aspasomal formulations

Drug content in the formulated Aspasomes was determined using a UV spectrophotometer.[23-25] The Formulated acyclovir aspasomal dispersion (1 mL) was pippetted out in a 10 mL volumetric flask

Table 1: Standard Calibration Curve of Acyclovir in PBS pH 7.4.

Concentration (µg/mL)	Absorbance
0	0
2	0.165±0.005
4	0.295±0.008
6	0.444±0.006
8	0.594±0.003
10	0.748±0.009

Values are mean±SD and n=3.

Table 2: Formulation Table for Acyclovir loaded Aspasomal Gel.

Formulation	Drug (mg)	Ascorbyl Palmitate (mg)	Cholesterol (mg)	Chloroform: Methanol (mL)	PBS pH 7.4 (mL)
F1	50	30	90	9:1	20
F2	50	30	60	9:1	20
F3	50	30	30	9:1	20
F4	50	60	90	9:1	20
F5	50	60	60	9:1	20
F6	50	60	30	9:1	20
F7	50	90	90	9:1	20
F8	50	90	60	9:1	20
F9	50	90	30	9:1	20

Total Volume=20 mL.

and the volume was made up to PBS pH 7.4 and analyzed at 256 nm wavelength using the equation,

$$\text{Drug Content} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Estimation of Vesicle Size (VS) and Poly Dispersibility Index (PDI)

The vesicle sizes of aspasomes were determined, by Nanotrac analyzer. The sample was diluted using milli-Q water for determination of Vesicle Size and PDI.

Determination of Percentage Entrapment Efficiency (%EE)

The ultracentrifugation technique determines the quantity of ACV entrapped inside the aspasomal system. The tube containing the aspasomal preparation was placed in centrifuge tubes and centrifuged for 3 hr at 4°C. Free acyclovir present in the supernatant was separated and diluted. The amount of ACV present was determined by using UV spectrophotometry at a wavelength of 256 nm.

%EE was calculated using the following equation,

$$\%\ EE = \frac{\text{Total Drug} - \text{Unentrapped Drug}}{\text{Total Drug}} \times 100$$

Morphological Evaluation of Aspasomes (TEM Analysis)

The morphology of Aspasomes was investigated using a high-resolution field emission gun transmission electron microscope, specifically the JEOL® JEM-2100F model operating at 200 kV.[26] To prepare the sample, a drop of aspasomes dispersion was carefully placed onto a carbon-coated copper grid. Subsequently, a solution containing 1% phosphotungstic acid dye was applied to the sample, allowing it to stain for a period of 2-3 min. Once the staining process was complete, the sample was left to air dry. Finally, the prepared sample was examined under the microscope, enabling detailed observation and analysis of the Aspasomes morphology. This method provides valuable insights into the structural characteristics of Aspasomes, aiding in the understanding of their properties and potential applications.

Experimental Design

Optimization of acyclovir loaded aspasomes was carried out by Experimental Design using Design Expert 13.0.11.0 version software.[23] A 3^2 randomized full factorial design was used for optimization of aspasomes. In this model, 2 factors were evaluated, each at 3 levels using Design Expert software to study the effect of critical independent variables/factors on the product quality attributes/response like Vesicles size and %Entrapment Efficiency

(Table 3). As Ascorbyl Palmitate and Cholesterol are the two most noteworthy factors which are believed to have significant effects on the Vesicle Size and % Entrapment Efficiency of aspasomes, they were selected as the independent factors.

Formulation Development using Design Expert, 3^2 Factorial Design

Total 9 formulations were formulated (F1-F9) and evaluated for vesicle size and % EE. Based on the results obtained, the formulation with lowest vesicle size and highest %EE was selected as the optimized formulation and was used for the preparation of Aspasomal Gel (Table 4).

Formulation of Aspasomal Gel

Acyclovir loaded aspasomal gel was prepared by soaking gelling agent (Carbopol 940) for 24 hr in measured quantity of Aloe Vera Juice (Table 5). The optimized aspasomal Formulation (F-8) was centrifuged for 30 min at 4°C.The pellets were separated from the supernatant and were dissolved in Aloe Vera juice and allowed to swell for 24 hr. The mixture was then subjected for stirring using magnetic stirrer. Triethanolamine was added dropwise to adjust the pH (neutral), Methyl paraben was added as a preservative and the resulting gel was then stored in air tight container for further analysis.[27]

Table 3: Amount of X_1 and X_2 generated by the software to be added in the formulation.

Run	(X_1) Amount of Ascorbyl Palmitate	(X_2) Amount of Cholesterol
1	30	90
2	30	60
3	30	30
4	60	90
5	60	60
6	60	30
7	90	90
8	90	60
9	90	30

Evaluation of Aspasomal Gel

pH

pH of the formulated aspasomal gel was determined using a digital pH meter, which was calibrated using standard pH stock solutions before use.[28,29]

Viscosity

Th viscosity of the formulated aspasomal gel was evaluated using the Brookfield CAP 2000+ Viscometer. Under ideal conditions of temperature and rpm the aspasomal formulation was checked for its optimal viscosity.

Spreadability

The therapeutic effect of the formulated cubosomal gel depends mainly on its spreading value. aspasomal gel spreadability testing was done using a Petri dish of 5 cm diameter in which 1 g of gel was placed at the centre. A 10 g of weight was placed on the top of an aluminium foil and allowed to rest for 5 min. A rise in diameter indicates a good spreadability.

In vitro Diffusion Study

Franz diffusion cell was used to determine the *in vitro* diffusion of acyclovir loaded aspasomal gel and marketed acyclovir gel.[30-32] 1 g of the formulated aspasomal gel was placed on the donor compartment. The receptor compartment was filled with the buffer (PBS pH7.4). The entire assembly was maintained at 37±0.5°C by using a magnetic stirrer. Cellophane membrane was used as the diffusion barrier. Aliquots of 1 mL were withdrawn

Table 4: Results of Vesicle Size, Entrapment Efficiency, Drug Content and PDI.

Formulation	Concentration	Vesicle Size (nm)	% Entrapment Efficiency	Drug Content (%)	PDI
F1	30:90	161.6±0.98	70.19±0.01	40.60±0.24	0.502
F2	30:60	98.21±1.54	80.80±0.04	61.87±0.01	0.442
F3	30:30	71.76±2.63	68.58±0.03	71.67±0.06	0.851
F4	60:90	193.5±0.87	72.31±0.01	64.32±0.04	0.442
F5	60:60	125.4±0.52	80.04±0.02	82.04±0.01	0.832
F6	60:30	84.97± 1.14	60.04±0.46	62.6±0.02	0.757
F7	90:90	223.6±2.03	79.01±0.05	78.53±0.04	0.780
F8	90:60	128.6±1.80	87.98±0.47	84.05±0.17	0.355
F9	90:30	75.35±1.58	72.90±0.03	60.87±0.01	0.267

Values are mean±SD and *n*=3.

Table 5: Formulation Table for Acyclovir Loaded Aspasomal Gel.

Ingredients	Quantity
Aloe vera juice	20 mL
Aspasomal suspension	Pellets (as per EE)
Carbopol 940	1.5%
Methyl paraben	0.01 - 0.05%
Triethanolamine	0.05%

at regular time intervals for 12 hr and immediately replaced with the same volume receptor medium to maintain sink condition. The total amount of drug released in 12 hr was calculated based on the absorbance of the samples using UV Spectrophotometer at 256 nm.

Ex vivo Diffusion Study

Franz diffusion cell was used to determine the *ex vivo* diffusion of acyclovir loaded aspasomal gel and marketed acyclovir gel.[30-33] 1 g of the formulated aspasomal gel was placed on the donor compartment. The receptor compartment was filled with the buffer (PBS pH7.4). The entire assembly was maintained at 37±0.5°C by using a magnetic stirrer. Rat skin was used as the diffusion barrier, facing the stratum corneum towards the donor compartment. Then, aliquots of 1 mL were withdrawn at regular time intervals for 12 hr and replaced immediately with the same volume receptor medium to maintain sink condition. The total amount of drug released in 12 hr was calculated based on the analysis of the samples using UV Spectrophotometer at λ_{max} 256 nm.

Release Kinetic Study

The drug release data revealed the pattern in which the drug release occurred. The release pattern was determined by various kinetic models such as zero order kinetics, first order kinetics, Higuchi model and Korsmeyer-peppas model.[34]

Stability Studies

Acyclovir-loaded Aspasomal formulation underwent stability evaluations following the ICH guidelines for 90 days at two distinct temperature conditions: 4±2°C and 25±2°C. The analysis focused on monitoring the vesicle size and %entrapment efficiency.

Statistical Analysis

The optimization of the Acyclovir-loaded Aspasomal formulation was achieved using Design-Expert® software (version 13, Stat-Ease Inc., MN, USA). To ensure the validity of the model and its constituent terms, analysis of variance (ANOVA) was applied to assess their statistical significance.

RESULTS AND DISCUSSION

Preformulation Studies

Identification test

Standard Calibration Curve for Acyclovir in PBS pH 7.4

The standard calibration curve of Acyclovir in PBS pH 7.4 was plotted by concentration versus absorbance. The graph displayed a straight-line (Figure 1) indicating linearity at λ_{max} 256 nm and followed Beer's range (2 to 10 µg/mL) (Table 1).

The correlation coefficient (R^2) obtained was 0.9994 with equation,

$$y=0.0739x+0.0046$$

Compatibility Studies by FT-IR Spectroscopy

To investigate the potential interactions between the drug and the excipients, absorption spectra of pure acyclovir and the physical mixture of acyclovir and excipients was measured. The characteristic N-H stretching vibrations at 3288.99 cm^{-1}, C=O stretching vibrations at 1716.65 cm^{-1}, C-O stretching at 1288.45 cm^{-1} and O-H stretching at 1408.04 cm^{-1} are visible in the Acyclovir spectra. The distinctive peaks of Acyclovir were intact in the FT-IR spectra of physical mixture suggesting the compatibility between the drug and the excipients Figures 2 and 3.

Characterization of Aspasomes

Estimation of Drug Content of Aspasomal Suspension

The drug content of all the formulations was found in the range of 40.6±0.24% to 84.05±0.17%. Cholesterol concentration had no significant effect on drug content whereas, increase in concentration of ascorbyl palmitate increased the drug content.[35] The results are as shown in Table 4.

Vesicle Size and Poly Dispersibility Index (PDI)

The vesicle size and PDI was determined by using Nanotrac Instrument. The vesicle size of acyclovir loaded aspasomes was found to be in the range of 71.76±2.63 nm to 223.6±2.03 nm (Table 4). It was observed that, concentration of ascorbyl palmitate and cholesterol had a positive effect on vesicle size which resulted in increase in vesicle size since lipid shapes into multiple layers as the concentration is increased making the vesicle bigger in size[36] and the same can be understood by the polynomial equation derived by Design-Expert® Software,

$$\text{Vesicle Size}=122.81+16.00\times A+57.77\times B$$

Where,

A=Concentration of Ascorbyl Palmitate,

B=Concentration of Cholesterol,

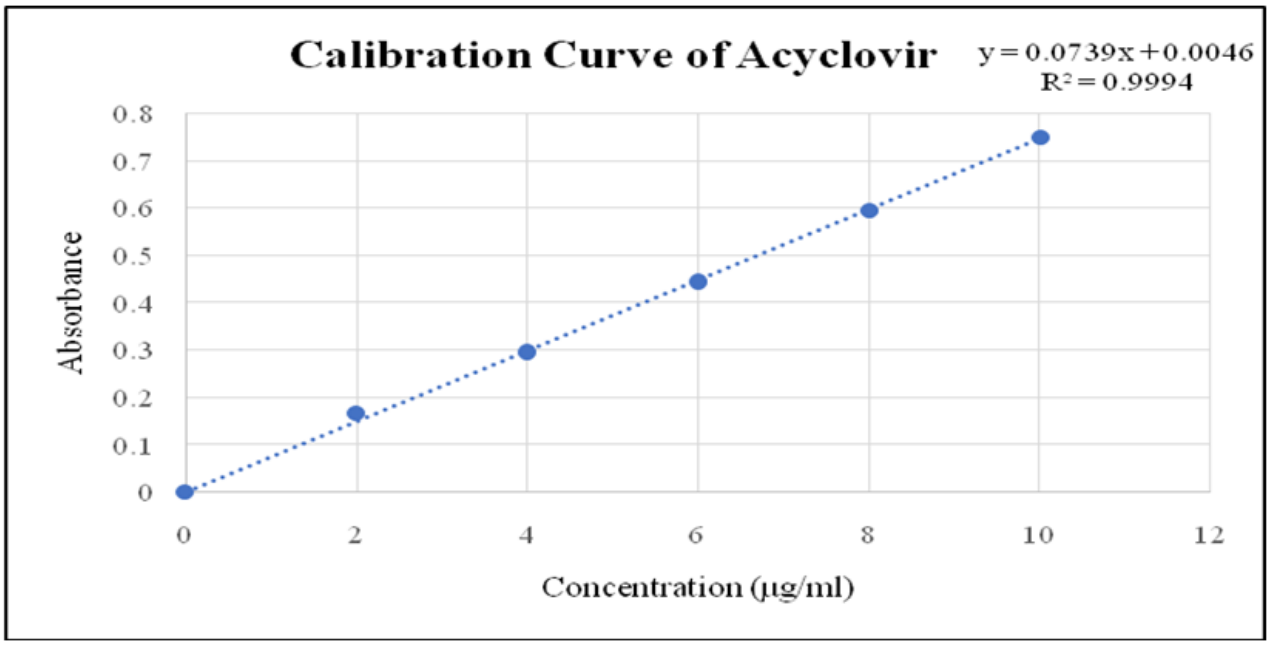

Figure 1: Calibration Curve of Acyclovir in PBS pH 7.4.

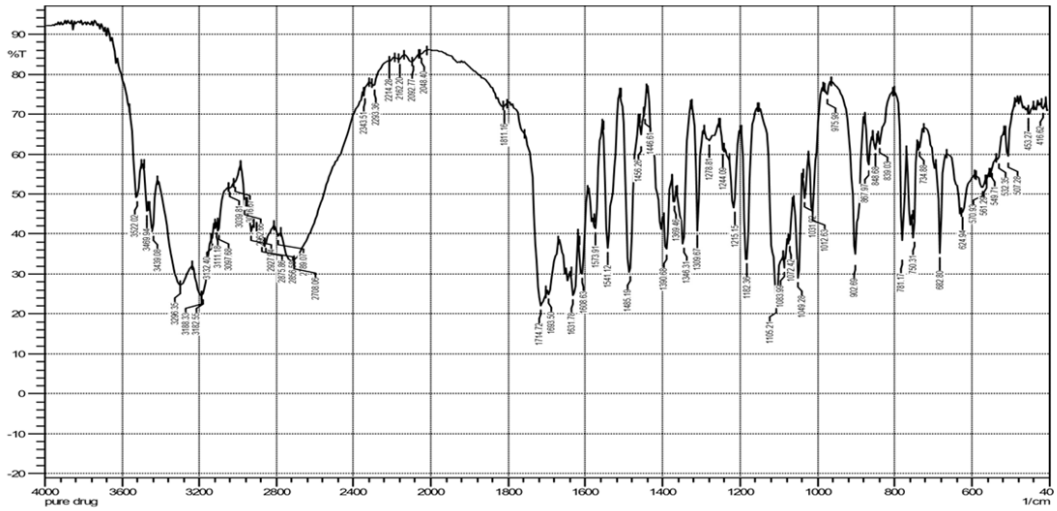

Figure 2: FTIR Spectrum of pure drug Acyclovir.

The graphical representation of the same can be seen in Figure 4.

Determination of % Entrapment Efficiency

The % EE of all the formulations was found in the range of 60.04±0.46% to 87.98±0.47%. It was observed that, concentration of ascorbyl palmitate and cholesterol exhibit a direct relation with the %EE of acyclovir. This was because the increased concentrations of ascorbyl palmitate and cholesterol made the drug difficult to enter into the vesicles.[36] The results were as shown in Table 4. The same can be understood by the polynomial equation derived by Design-Expert® Software,

$$\%\text{Entrapment Efficiency} = 79.09 + 3.39 \times A + 3.33 \times B$$

Where,

A=Concentration of Ascorbyl Palmitate,

B=Concentration of Cholesterol,

The graphical representation of the same can be seen in Figure 5.

Morphological Evaluation of Aspasomes (TEM Analysis)

Transmission Electron Microscopy (TEM) images of the prepared aspasomes confirm the morphology and structure. It reveals that the aspasomal vesicles were spherical in shape, well isolated from each other and in the nano size range[36] (Figure 6).

Table 6: Release Kinetics Study.

Formulation	Zero Order (R^2)	First Order (R^2)	Higuchi Matrix (R^2)	Peppas Model		Best Fit Model
				(R^2)	(n)	
Acyclovir Aspasomal Gel (F8)	0.9526	0.9525	0.8102	0.9920	1.8560	Peppas

Table 7: Stability Study.

Evaluation Parameters	Before	Room Temperature (25°C±2°C)		Refrigerated Condition (4°C±2°C)	
	Day 1	30 Days	90 Days	30 Days	90 Days
Entrapment Efficiency (%)	87.98±0.47	83.42±0.38	79.36±0.72	85.55±0.26	83.12±0.34
Vesicle Size (nm)	128.6±1.80	132.8±2.01	146.3±1.56	129.2±1.32	133.1±1.89

Values are mean±SD and n=3.

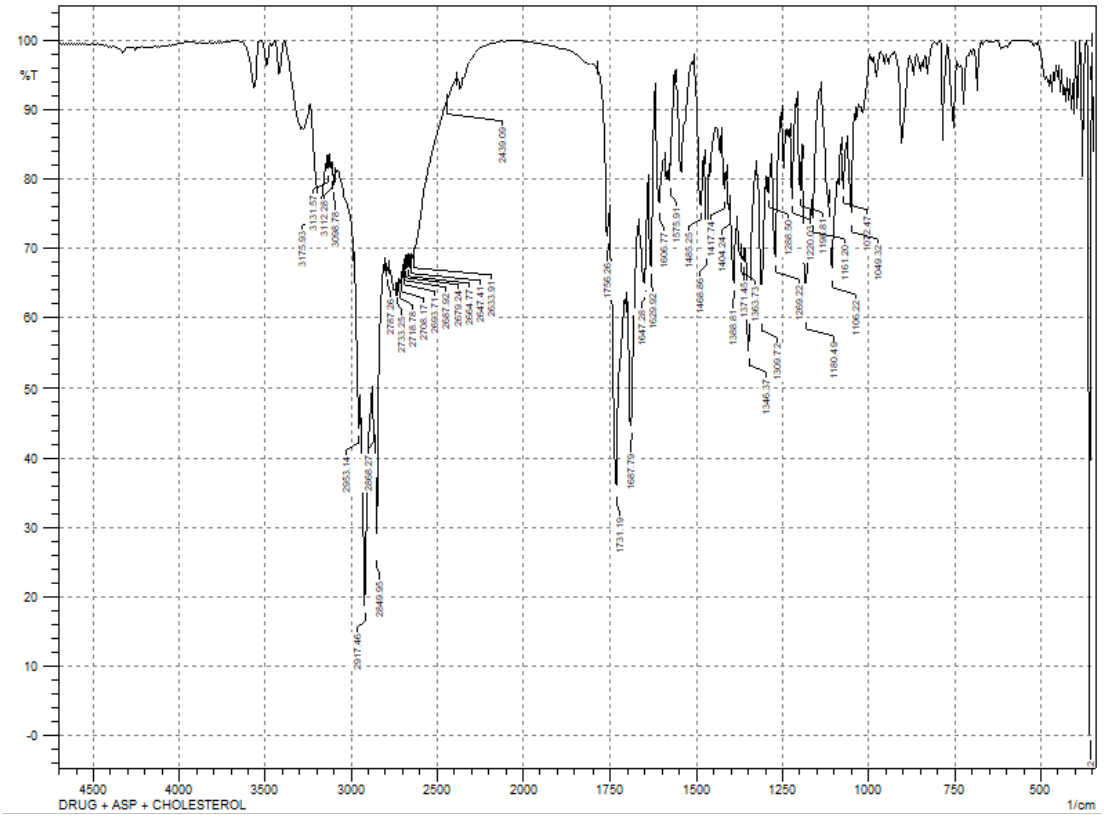

Figure 3: FTIR Spectrum of physical mixture of Acyclovir and Excipients.

Characterization of Acyclovir loaded Aspasomal Gel

pH

The pH of the prepared acyclovir loaded aspasomal gel was found to be 6.34±0.126 and that of marketed acyclovir gel was found to be 7.01±0.113.

Viscosity

The viscosity of gels was determined by using Brookfield Viscometer. Viscosity of Acyclovir loaded Aspasomal Gel was found to be 1557±0.213 cps and that of marketed Acyclovir Gel was found to be 1438±0.198 cps.

Spreadability

The spreadability of acyclovir loaded aspasomal gel was found to be 6.9±0.163 gm.cm/sec and that of marketed acyclovir gel was observed to be 6.2±0.201 g.cm/sec. Carbopol 940 used as the gelling agent enhances gel spreadability by thickening formulations, suspending particles evenly and exhibits shear-thinning behaviour.[36]

In vitro Diffusion Study

Franz diffusion cell was used to determine the *in vitro* diffusion of acyclovir loaded aspasomal gel and marketed acyclovir gel

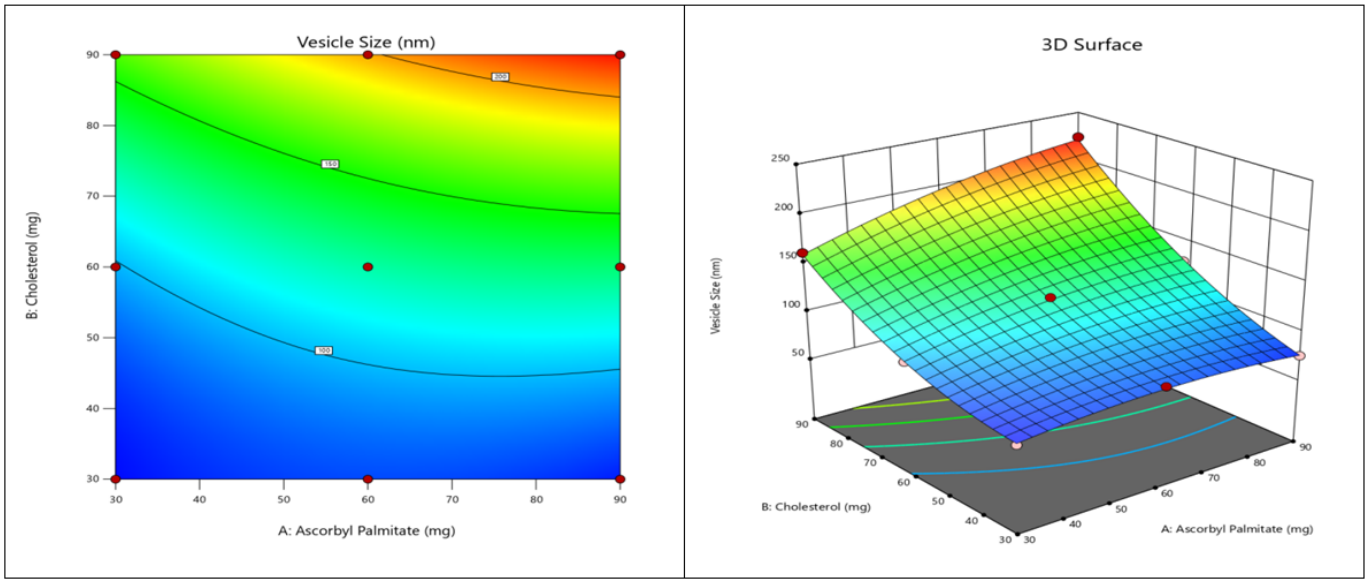

Figure 4: Contour plot and Response surface plot showing the effect of concentration of ASP (X1) and Cholesterol (X2) on Vesicle Size.

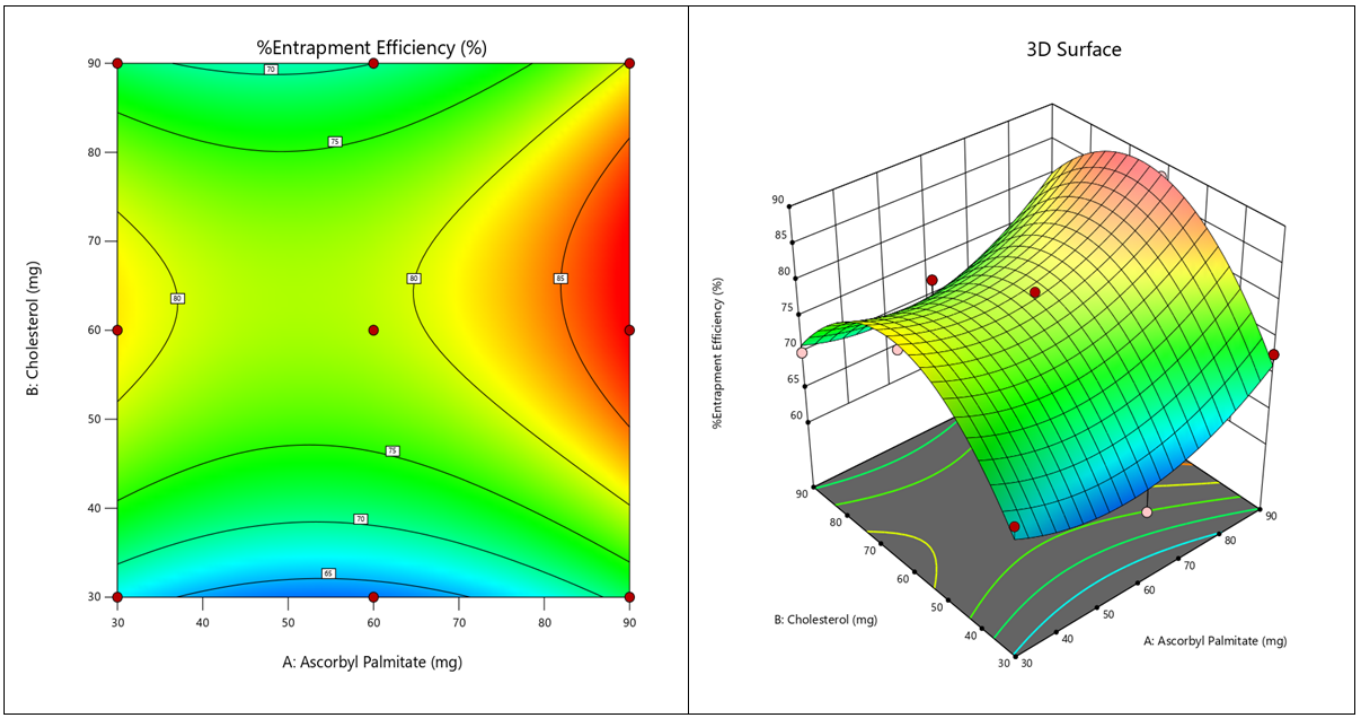

Figure 5: Contour plot and Response surface plot showing the effect of concentration of ASP (X1) and Cholesterol (X2) on %EE.

using cellophane membrane. The total amount of drug released in 12 hr was calculated. The result revealed that 0.715±0.11% to 87.48±0.42% amount of acyclovir was released from the aspasomal gel. Marketed acyclovir gel released 0.661±0.51% to 34.077±0.43% of the drug which clearly depicts the enhanced drug diffusion of optimized aspasomal gel. It was observed that the diffusion of drug increased as the concentration of ascorbyl palmitate and cholesterol was increased from 30% to 90% v/v, but on further increase in the concentrations (50% v/v), the drug release dropped significantly. Ascorbyl Palmitate and Cholesterol considerably retarded drug diffusion when the concentration was significantly increased; this was because increased concentrations can potentially modify the structure and properties of Aspasomes. This might also alter the integrity of Aspasomal membrane.[37] Hence, 50% v/v was considered as the optimum concentration (Figure 7).

Ex vivo diffusion Study

The *ex vivo* diffusion study of formulated acyclovir aspasomal ael and marketed ayclovir gel was studied for 12 hr, which revealed that acyclovir from the aspasomal gel was released in a controlled manner. As it was observed that at the end of 12 hr, aspasomal

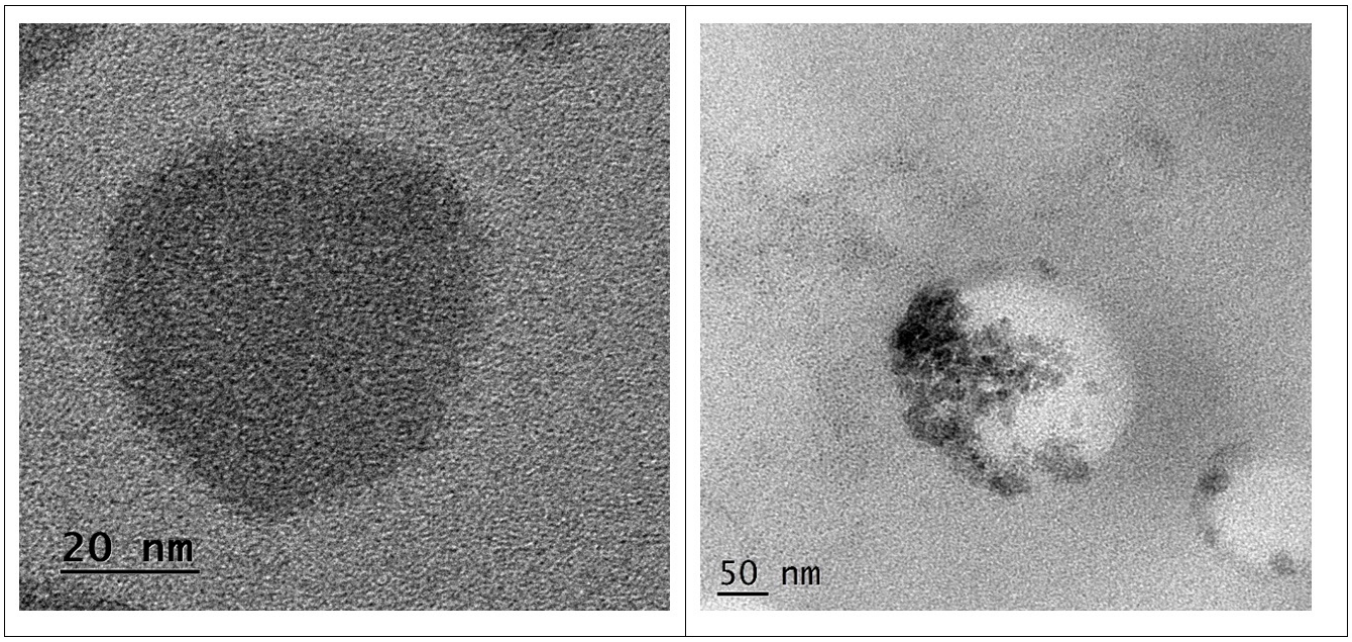

Figure 6: Morphological (TEM) Images of the formulated Aspasomes.

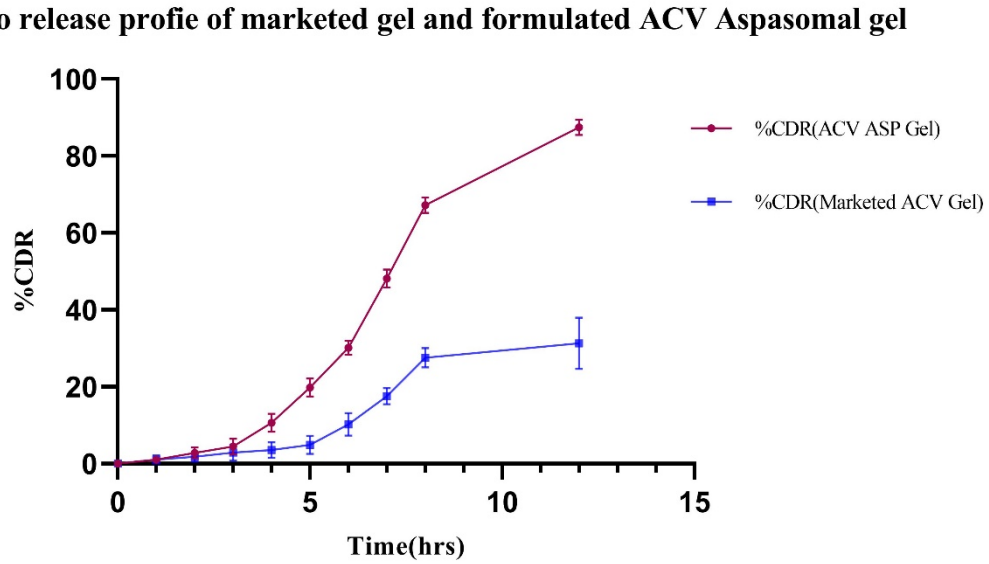

Figure 7: *In vitro* release profile of Marketed gel and formulated ACV Aspasomal gel.

gel released 63.56±0.36% of acyclovir while only 30.64±0.62% of acyclovir was released from the marketed gel. This effect can be due to the structure and size of aspasomes and the presence of ascorbyl palmitate which is a core ingredient in the formulation of aspasomes, gives a synergistic effect to the diffusion property of nanovesicles, which helps the aspasomal gel to release more drug in comparison to marketed gel at the end of 12 hr. Additionally, the optimal concentration of cholesterol contributed to the increased penetration by enhancing the elasticity of the lipid vesicles. By applying aspasomal gel to the skin, the gel diffuses from the dry stratum corneum to a deep moist layer as a result of the osmotic gradient. This can be facilitated by addition of an occlusive gel base because occlusion increases diffusion by reducing transepidermal water loss. Occlusive gel base like aloe vera gel base, which has been used in the formulated aspasomal gel enhances the diffusion property of the formulated acyclovir aspasomal gel compared to marketed acyclovir gel (Figure 8).[37]

Release Kinetic Study

The release of Acyclovir loaded Aspasomal gel formulation and marketed Acyclovir gel followed zero order kinetics pattern with highest R^2 value of 0.9256 which states that release pattern was through diffusion (Table 6).

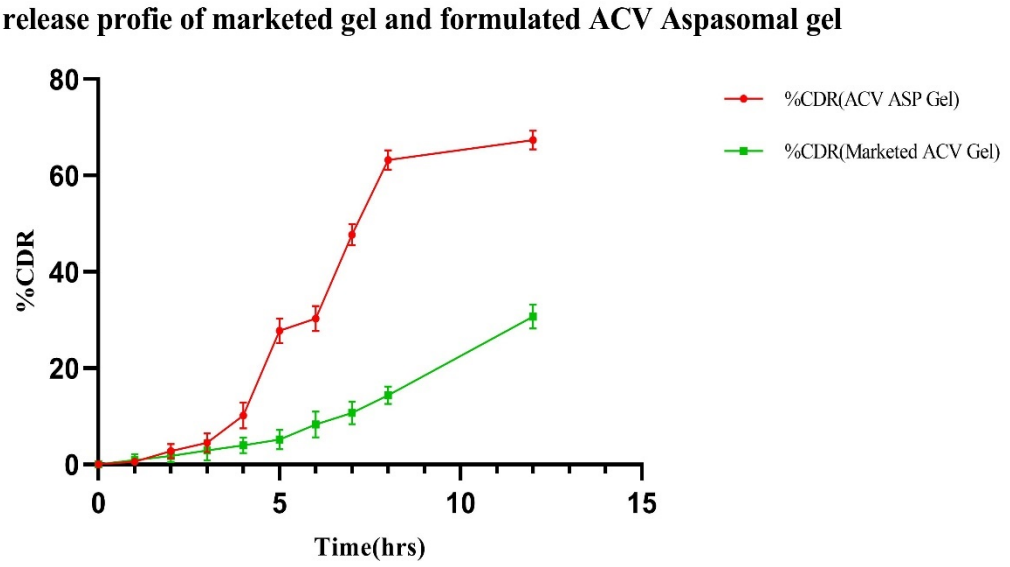

Figure 8: *Ex vivo* release profile of Marketed gel and formulated ACV Aspasomal gel.

Stability Study

The Vesicle Size and %Entrapment Efficiency were evaluated on day 1, after 30 days and followed by 90 days of different storage conditions. It was observed that there were slight changes in Vesicle Size and %Entrapment Efficiency during the storage conditions. But, as the changes were within the acceptable limits, the formulation is said to be stable (Table 7).

CONCLUSION

The present study was been satisfactorily performed and an attempt was made to formulate acyclovir loaded aspasomal gel for transdermal delivery by enhancing permeability of the drug. The FTIR spectra revealed that there was no interaction between drug and excipients and was found to be compatible with each other. The vesicle size of prepared Aspasomes was found in the range of 71.76±2.63 nm to 223±2.03 nm. Based on the results obtained, F8 formulation was considered as the optimized formulation as it showed smallest vesicle size of 128.6±1.8 nm, highest % drug content of 84.05±0.17% and highest % entrapment efficiency of 87.98±0.47%. Thus, F8 formulation was considered as optimized formulation for the preparation of Aspasomal gel. The Aspasomal gel was prepared by incorporating the optimized Formulation (F8) into the Aloe Vera gel base. The *In vitro* drug diffusion study showed the higher percent cumulative drug release from formulation F8 i.e., 87.48±0.42% at the end of 12 hr, which was prepared by using Carbopol 940 (1.5%). The release of Formulation (F8) ACV loaded ASP gel followed zero-order kinetic pattern. The optimized (F8) Acyclovir loaded Aspasomal topical gel was used as an effective Anti-Viral agent for HSV Treatment. Aloe Vera gives moisturizing effect and helps to heal the skin rapidly and Ascorbyl Palmitate itself has antioxidant activity. Thus, Aspasomes are considered as promising carrier for transdermal drug delivery.

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FUTURE SCOPE

Although the extensive research unveiled numerous promising outcomes, encompassing the formulation, *in vitro* and *ex vivo* evaluations for the effective management of Herpes, the need for further *in vivo* studies and clinical studies remains crucial to establish the definitive efficacy of this treatment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PDI: Polydispersity Index; **HSV:** Herpes Simplex Virus; **ACV:** Acyclovir; **ASP:** Ascorbyl Palmitate; **TEM:** Transmission Electron Microscopy.

SUMMARY

Acyclovir loaded aspasomes were formulated to enhance the delivery of ACV by preparing vesicles comprising of ascorbyl palmitate and cholesterol. These vesicles were used to enhance permeability of acyclovir (BCS class IV) for better transdermal delivery. The Aloe Vera gel, rich in its healing and anti-inflammatory properties, served as the basis for the

formulation. Aspasomes provided prolonged and targeted delivery of acyclovir providing a promising topical formulation, with reduced side effects.

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