Available online on 15.09.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Research Article

Formulation and Evaluation of Acyclovir Loaded Transferosomal Gel for Transdermal Drug Delivery

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Article Info:

Article History:

Received 04 July 2024
Reviewed 10 Aug 2024
Accepted 03 Sept 2024
Published 15 Sept 2024

Cite this article as:

Gosavi AA, Thorat PA, Mulla JAS, Formulation and Evaluation of Acyclovir Loaded Transferosomal Gel for Transdermal Drug Delivery, Journal of Drug Delivery and Therapeutics. 2024; 14(9):122-130 *DOI: http://dx.doi.org/10.22270/jddt.v14i9.6786*

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Abstract

Background: A carrying structure for targeted transdermal drug delivery is a transferosome. These unique liposomes are made of an edge activator and phosphatidylcholine. The most common antiviral drug is acyclovir, a synthetic nucleotide nucleoside analog derived from guanine. It works well to cure the varicella-zoster virus and the virus that causes herpes simplex (HSV), primarily HSV-1 and HSV-2. But its skin permeability is minimal. Therefore, this work aimed to use transferosomes to prepare acyclovir so that it could pass through the skin's barrier function.

Objective: This study uses a 3^2-factorial factorial design to develop a transferosomal gel containing acyclovir through thin-film hydration method for painless acyclovir delivery services for skin disease treatment.

Material and Methods: The independent variables are the amount of phospholipid (X_1) and tween 80 (X_2), while the dependent variables are particle size (Y_1) and percentage entrapment efficiency (Y_2). To create an ideal formulation, the produced transferosomes were assessed for particle size, *in vitro* drug release amount, and entrapment efficiency (EE%). A Carbopol 934 gel basis was prepared using the optimized acyclovir transferosome formulation, and its drug concentration, pH, spreadability, viscosity, and stability were assessed.

Results: With small particles ranging from 176.6 to 324.4 nm, the produced acyclovir transferosomes had a high EE% range from 66.34 to 76.42 %. According to the in vitro release study, there is a negative correlation between in vitro release and EE%. The formulation TF5, including 1%w/w of carbopol 940, provides a superior profile of drug absorption in vitro. Consequently, acyclovir can enter the skin as transferosomes and cross the stratum corneum barrier.

Conclusion: Acyclovir can be transformed into a transfersomal gel, which will improve antiviral efficacy, get over the skin's protective layer, prevent adverse oral reactions, and eventually improve patient adherence.

Keywords: Transferosome, Transdermal drug delivery system, Acyclovir, 3^2 Fractional factorial design, Carbopol 934

INTRODUCTION

In addition to its convenience and safety, transdermal medication administration is a fascinating option. Compared to traditional drug delivery methods, they offer a number of benefits, including the ability to stop the first pass of metabolism, an ongoing and prolonged period of action, a reduction in unwanted side effects, the ability to utilize medications with short half-lives, an improvement in pharmacological and physiological response, the ability to prevent changes in drug concentration, within and inter-patient variations, and, above all, patient convenience[1].

Drug delivery systems based on vesicles are an effective and successful way to deliver prescription drugs to the right places. The Transfersome falls under the vesicular systems category[2].

Bilayer vesicles with remarkable flexibility are called transferosomes. They can readily pass through the stratum corneum's intracellular lipids layer and beyond the skin's barrier function. Transferosomes penetrate the skin's outer layer, which is dry, after skin application and enter a highly hydrated layer along the gradient of osmotic pressure. The lipid in the corneum stratum is more permeable and the vesicles may penetrate the layer deeper due to a surfactant in their structure. Acyclovir is the most frequently utilized antiviral drug[3]. Topical drug delivery refers to the application of a drug topically for a targeted effect; systemic medication administration through the skin is possible with dermal drug delivery devices (TDDS)[4].

The most often prescribed antiviral medication is acyclovir. It is an artificially produced purine nucleoside analog that is made from guanine. HSV-1 and HSV-2 are the two types of the viruses that cause herpes simplex (HSV) and have successful treatments. Acyclovir is classified as a category III drug under the drug class

system because of its excellent solubility and low permeability[5].

A thorough description of acyclovir, including its pharmacokinetics, pharmacodynamics, and several delivery methods, is given by Khan & Kumar (2010)[6]. Recent developments in the formulation and delivery of acyclovir, including innovative drug delivery technologies, are covered by Ali & Hwang (2021)[7]. Khan et al. (2018) concentrates on using nanoparticles to enhance acyclovir distribution and effectiveness[8]. The usefulness of liposome-encapsulated acyclovir in the treatment of herpes simplex virus infections is examined by Wang et al. in 2022[9]. Yao & Zhang (2019) investigate ways to use solid dispersion techniques to increase the solubility and bioavailability of acyclovir[10]. An article published in 2020 by Sanghavi & Mody describes how acyclovir nanosuspensions have been developed to improve oral bioavailability[11]. Afzal Hussian et. al. (2023) investigated the mechanisms underlying the permeability profiles of acyclovir across artificial membranes using vesicular ethosomes and elastic liposomes[12].

Acyclovir distribution for the treatment of skin ailments. Acyclovir is prepared using transferosomes to allow it to cross the barrier function of the skin. The use of transferosomes as vascular nano-carriers has been selected and investigated as a viable approach for improving oral health and transdermal drug delivery.[3]

MATERIALS AND METHODS

Received a complimentary sample of acyclovir from Swapnroop Chemicals in Aurangabad. The supplier of phospholipid was Scientific Lab Good Chem Industries in Mumbai. Purchased from Loba Chemicals, Mumbai, were a solution of chloroform, cholesterol, tween 80, propylene glycol, and triethanolamine. We bought Carbopol 934 from Molychem in Mumbai. When necessary, freshly manufactured demineralized and doubly distilled water was used. In every other case, scientific-grade reagents and chemicals were used.

Preparation of transferosomes

Step 1: Formation of Lipid Film

The lipid, edge activator, and drug were dissolved in 30 millilitres of methanol and chloroform (3:1) in a 100 millilitre flask with a circular bottom (Table 1). The flask was attached to a revolving evaporation unit (Superfit, India) that rotated while immersed in a 60°C water bath. Until the solvent that was used had totally drained and a lipid layer had developed on the flask walls, this process was repeated. In order to ensure that the remaining solvent was completely removed, the flask was kept overnight in a vacuum desiccator.

Step 2: Hydration is added to the created film

Once combined with a pH 7.4 buffered phosphate solution, the dried film became restless for half an hour more, simulating rotational vacuum evaporation, until the fat film was fully hydrated.

Step 3: Tiny vesicles start to develop

After being removed from the flask, the transferosomes were sonicated for 15 minutes at 50 Hz in a bath sonicator[13].

Table1: Preparation of Acyclovir loaded Transferosomes

Sr. No.	Formulation code	Acyclovir (mg)	Soya lecithin (mg)	Tween80 (mg)	Choles-Terol (mg)	Chloroform :Methanol (2:1) (ml)
1	F1	50	80	15	10	30
2	F2	50	80	20	10	30
3	F3	50	85	10	10	30
4	F4	50	85	15	10	30
5	F5	50	90	20	10	30
6	F6	50	85	20	10	30
7	F7	50	80	10	10	30
8	F8	50	90	10	10	30
9	F9	50	90	15	10	30

Experimental Design

The statistical efficiency of the design of the experiment was examined using the software package Design Expert 12. The central composite design was the most often employed reaction surface technique. For this experiment, nine trials using a three-level design were carried out. Using a 3^2-design, the effects of independent variables like Tween 80 (X2) and soy lecithin (X1) quantities were analyzed alongside dependent variables like particle size (nm) and entrapment efficiency (%EE). An ANOVA was used to evaluate the model's significance (Tables 2 and 3).

Table 2: Independent variables with their coded levels and concentration of phospholipid and surfactant

Coded values level	Independent Variables	
	X_1 Amt. of Soya lecithin (mg)	X_2 Amt. of Tween 80 (mg)
-1	80	10
0	85	15
+1	90	20

Table 3: Design matrix for experimentation

Formulation	Factor 1	Factor 2
	X_1: Soya Lecithin mg	X_2: 80 mg
F1	80	15
F2	80	20
F3	85	10
F4	85	15
F5	90	20
F6	85	20
F7	80	10
F8	90	10
F9	90	15

Characterization of Transferosomes

Particle Size

Size was measured using a Zetasizer 300HS. Samples were mixed with distilled water and then measured at 25ºC. The diameter was calculated using the self-correlation coefficient based on the transferosomes' light-scattering brightness[5,14-17].

Zeta potential

Using a zeta sizer, the average particle size of the transferosome and its Zeta Potential (ZP) were determined. The mean particle size is one important component that regulates the degree of absorption into the skin. According to Helmholtz Smoluchowsky, the zeta potential was ascertained by measuring the electrophoretic mobility of the samples. Zeta potential on a huge bore measurement cell with an electromagnetic intensity of 20 V/cm was evaluated using Zetasizer. The samples were diluted to a resistivity of 50 l S/cm using 0.9% NaCl[18-22].

The polydispersity index (PDI)

Gauges a sample's degree of heterogeneity according to its size; polydispersity can result from sample agglomeration. The dynamical dispersion of light microscopy (DLS) method can be used to determine PDI. In terms of PDI, less than 0.1 denotes homogeneity and less than 0.4 denotes heterogeneity[23].

Entrapment efficiency (EE)

To calculate the entrapment efficiency, the quantity of free drug that remained non-entrapped in a solution of water was assessed. Each Eppendorf tube was filled with approximately 1 millilitre of a drug-loaded the transfersomes dispersion, and then the tubes were spun at 10,000 rpm for 30 minutes. The transfersomes and the encapsulated drug were maintained apart at the bottom of the tubes. As a control sample, plain transferosomes devoid of acyclovir were employed and handled similarly. The amount of free drug was determined by measuring the effluent's ultraviolet (UV) absorbance at 253 nm[24].

Transferosomal Gel Preparation:

After carefully weighing Carbopol 934 (1%w/v), 80ml of water that had been double-distilled was added to a beaker. The mixture was stirred continuously for an hour at 800 rpm, and then 10 milliliters of propylene glycol (PG) were added. The gel's volume was adjusted to 100 ml, and it was sonicated for 10 minutes on a bath sonicator to eliminate air bubbles. In order to attain the necessary drug concentration in the gel basis, 2% w/w of transferosomal preparation acyclovir was added. Triethanolamine was used to modify the gel bases final pH to 6.8 [25].

Transferosomal Gel Characterization

pH

Using a pH meter, the produced transferosomal gels pH was determined. The necessary number of gels has been incorporated into 10 ml of distilled water to create a homogenous solution. After immersing the electrode in suspensions, pH meter readings were recorded[26].

Viscosity Measurement:

A viscometer from Brookfield and associates DVE was used to measure the consistency of the gel. Spindle number S64 was operated at 12 rpm for 10 seconds at 37°C[27].

Spreadability

Spreadability can be evaluated using two glass slides. A second prepared gel slide, weighed down by 10 g, is placed on top of a 500 mg produced gel slide. After giving the gel, a minute to spread, the length of gel that has covered the top of the slide is measured using a scale. By measuring the amount of time, it took to move the two slides apart in seconds, the spreadability of each slide was evaluated using the formula (equation 1)[27]:

$$S = \frac{m \times l}{t} \quad \ldots\ldots (1)$$

Where:

m -Weight attached to higher slide

S - Spreadability in g.cm / sec

l - glass slides length

t- time in seconds

Drug Content:

To dissolve the medication in the methanol, a 50millilitre volumetric flask holding one milligram of each formulation, or around 40 mg of each drug, was filled with a solution of diluted, and shaken. The mixture was filtered with Whatman filter paper. Pipetting out 0.1 ml of the filter, 10 ml of methanol was combined with it. A UV Spectrophotometer was utilized to analyze the drug's composition[28].

$$\%\ Drug\ Content = \frac{Amount\ taken}{Amount\ obtained\ after\ centrifugation} \times 100 \quad \ldots\ldots (2)$$

***In vitro* Drug Release**

The device is made up of a cylinder of glass with open ends. Adhered with glue to both sides of the cylinder is a dialysis membrane that has been steeped in water that was filtered for an entire day before to usage. To create the receptor compartment, the cell is surrounded with gels containing 10 mg of drug (donor chamber) and submerged in 100 ml of 7.4 pH PBS with 10% v/v alcohol (to maintain sink state). Throughout the system, the lower end of each gel-containing cell is positioned to be no more than 1-2 mm below the diffusion medium's surface. After heating the medium to 37 ± 0.5°C, it was agitated using a magnetic stirrer. Occasionally, a portion (5 ml) is removed from the designated chambers and replaced with an equal volume of fresh buffer. With the use of a UV-visible spectrophotometer, the samples were examined. Three duplicates of each test run were carried out[29-30].

Release kinetics

Various kinetic models were used to fit the kinetics and mechanism of acyclovir release from transferosomal gel during an *in vitro* drug release investigation. The following kinetic models were investigated: The zero order model (equation 3), the first order model (equation 4) and the Higuchi model (equation 5)[31].

$Q = K_0 t$ ..... (3)

$\log Q_{10} - \log Qt = K_1 t/2.303$ ..... (4)

$Q = K_2 t^{1/2}$ ..... (5)

RESULTS AND DISCUSSION

Experimental Design

The 3^2-factorial design method was taken into consideration, wherein two parameters at three distinct levels affected the particle size and entrapment efficiency. To neutralize the effects of unimportant or bothersome variables, every experiment was run in a different order every time. Utilizing Design Expert software, the experimental design findings were examined. This analysis yielded a wealth of insightful data and confirmed the value of statistical design in experimentation. The results of the chosen variables that are independent, such as the amount of EA and acyclovir on the size of the particles and Acyclovir entrapment efficiency, are clearly shown in Table 3, which displays different combinations of variables that are independent, and the resulting impacts on the variable that is dependent.

Particle size

The experimental data was entered into a quadratic polynomial model using the design-expert tool. The equation for the ideal particle size in terms of the factor codes was discovered to be:

$$Particle\ size = 33.47 - 858*A + 11.93*B - 8.40*A*B - 42.95*A^2\ 95.00*B^2$$

In this case, A, A 2, and B2 are significant model terms with the p-value less than 0.05. The coefficient's R^2 value of 0.9716 indicated that the independent variables accounted for 97.16% of the variation in the responses. Adeq. precision determines the signal-to-noise ratio. The ratio ought to be more than 4. A proportion of 6.24 indicate a strong enough signal. This model can be used to navigate the design area. The observed pattern of an initial increase followed by a reduction in particle size with varying amounts of phospholipid and Tween 80 typically reflects the presence of an optimal concentration for each component. Deviations from this optimal range can lead to larger particle sizes due to instability, aggregation, or poor bilayer formation. Once the optimal range is achieved, further increases or decreases in these components can lead to improved stabilization and formation of smaller, more uniform vesicles (Figure 1a, 2a).

Entrapment efficiency

Using the design-expert program, the experimental results were plugged into the quadratic polynomial

model to determine the optimum entrapment efficiency. The equation for this efficiency was found to be:

Entrapment efficiency = 69.77+081.83*A+2.71*B+66 *A^2*B^2+2.23*A*B

In this case, A, A 2, and B2 are significant model terms with the p-value less than 0.05. The estimating factor R^2 value of 0.8010 indicates that 80.10% of the variation in the responses was explained by the independent variables. A proportion of 69.77 indicate a strong enough signal. This idea can be used to navigate the design space. Increasing the amount of soy lecithin enhances the structural integrity of the transferosome bilayer, while Tween 80 improves the flexibility and overall stability of the vesicles. The combination of these effects at the higher lecithin concentration results in a more efficient encapsulation of the drug, as the vesicles can better retain and protect the encapsulated substance (Figure 1b, 2b).

Table 4: Design matrix and responses

			Factor 1	Factor 2	Response 1	Response 2
Formulation	**Std**	**Run**	**X_1: Soya Lecithin**	**X_2: Tween 80**	**Y_1: Particle size**	**Y_2: Entrapment Efficiency**
			mg	mg	nm	%
F1	5	1	80	15	316.2	66.48
F2	3	2	80	20	214.5	70.38
F3	7	3	85	10	228.3	67.26
F4	9	4	85	15	324.4	71.32
F5	4	5	90	20	193.1	76.42
F6	8	6	85	20	257.7	72.28
F7	1	7	80	10	176.6	69.22
F8	2	8	90	10	188.8	66.34
F9	6	9	90	15	273.9	68.23

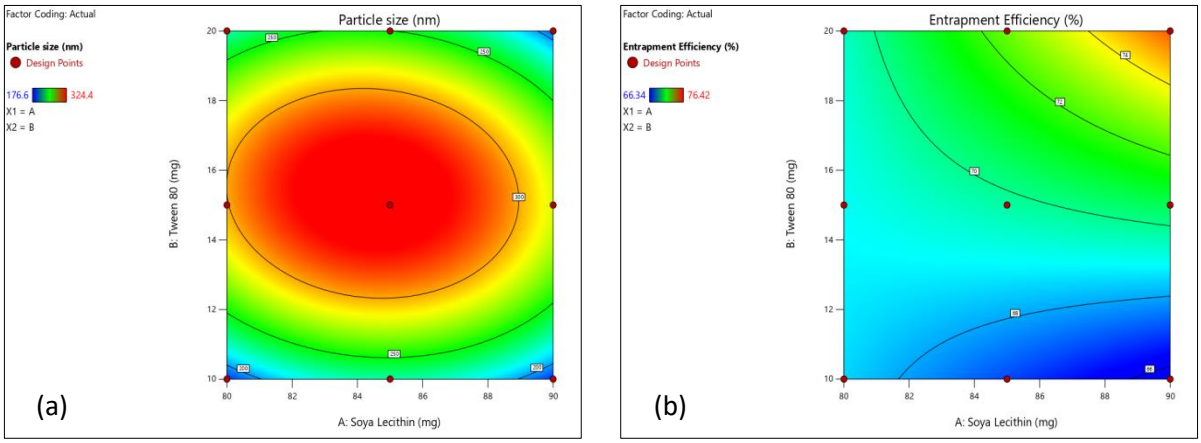

Figure 1: Particle size and entrapment efficiency in 2D contour plots

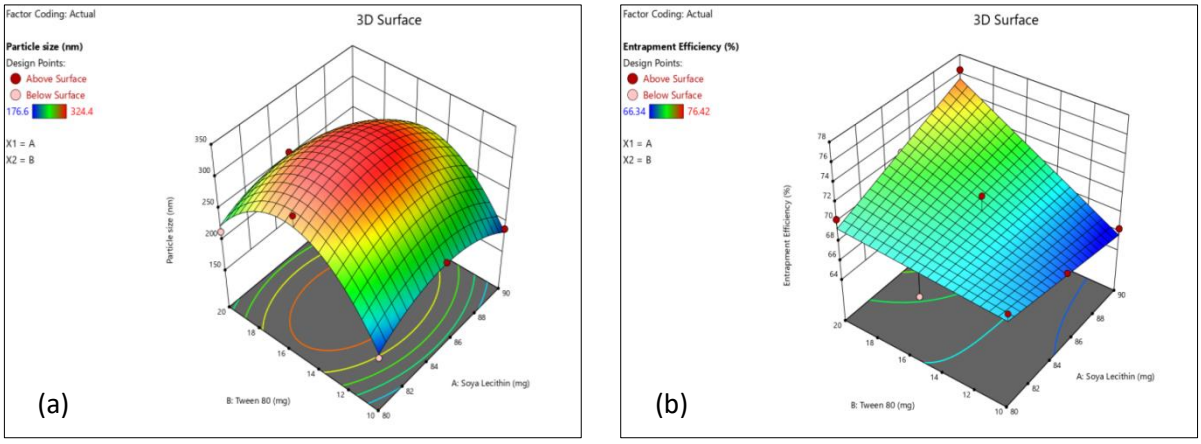

Figure 2: 3D response surface plot for particle size and entrapment efficiency

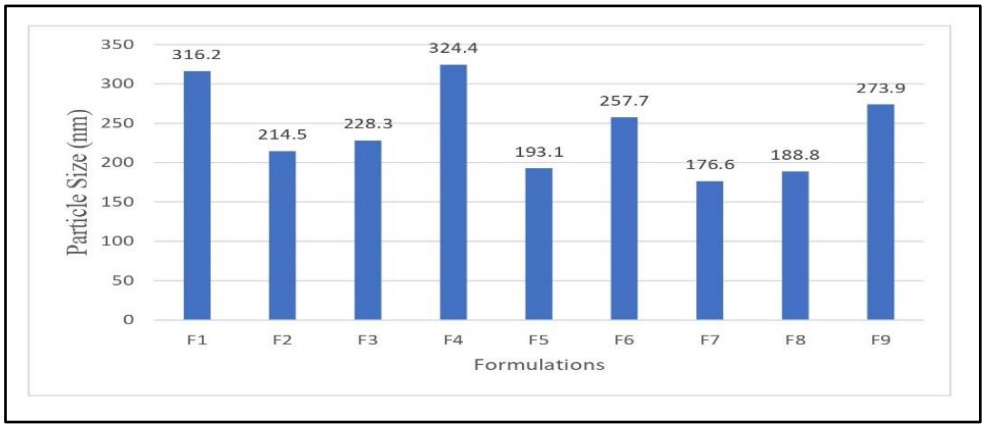

Figure 3: Particle size graph of Acyclovir Transferosomes (All Formulations)

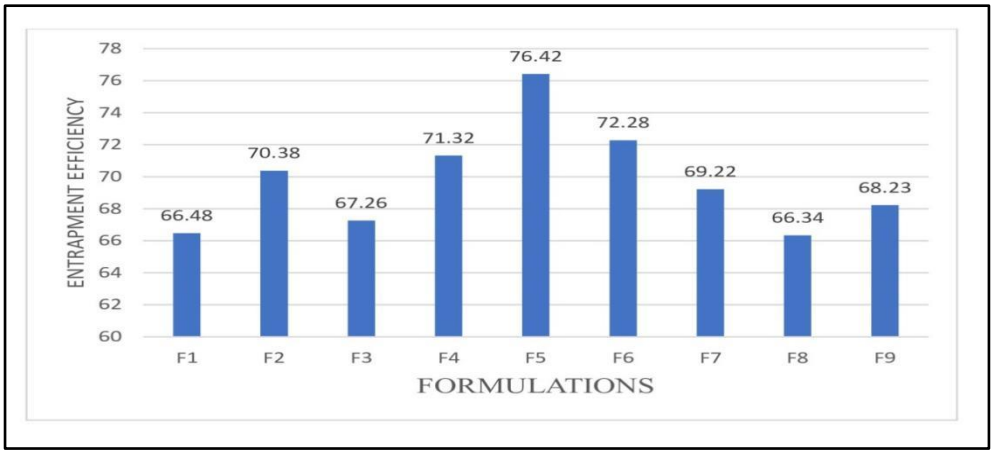

Figure 4: Entrapment efficiency graph of Acyclovir Transferosomes (All Formulations)

Table 5: Optimized formula after post Analysis

Formulation	Soya lecithin	Tween 80
F5	90	20

Zeta potential

The measurement of the zeta potential allows predictions about the stability of transferosome formulation. The zeta potential of optimized formulation was observed to be -26.4 mV, indicating greater stability. (Table 6, Figure 6).

Table 6: Particle size and zeta-potential results of batch F5 of optimized Acyclovir transferosome formulation

Optimized Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment efficiency
Acyclovir Transferosomes	193.1	0.28	-26.4	76.42

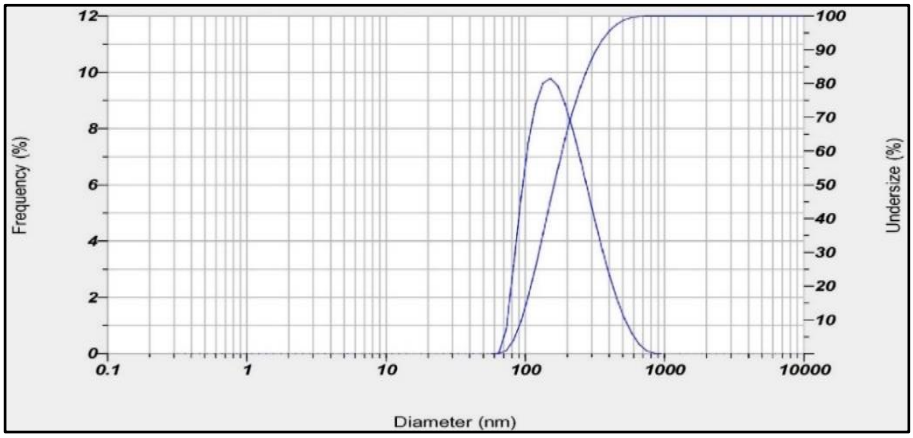

Figure 5: Particle size of optimized transferosomal drug delivery system F5

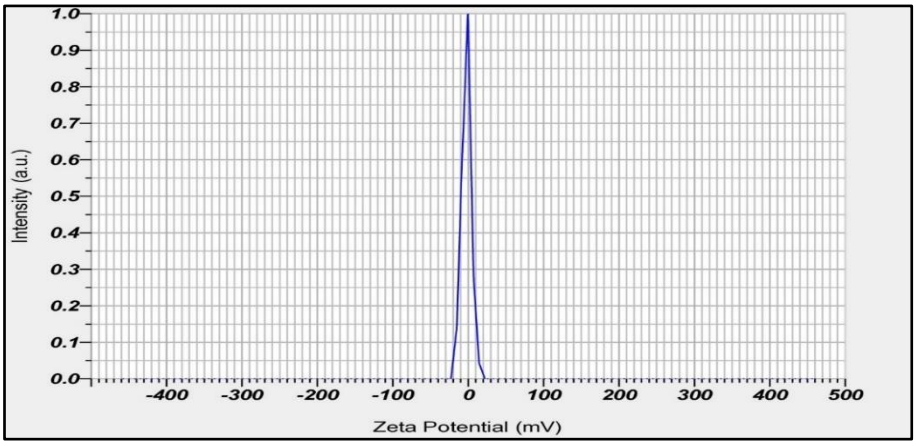

Figure 6: Zeta potential of optimized drug delivery system F5

Characterization of Transferosomal Gel

pH

A transferosomal gel's pH is significant since it influences the gel's stability and skin compatibility. Since this pH range is so close to the skin's natural pH, it is generally preferred for skin care products. It was discovered that the pH of the Acyclovir Loaded Transferosomal Gel was 6.8.

Viscosity

The gel's thickness or flow resistance is gauged by its viscosity. A transferosomal gel needs to have the right viscosity for stability and ease of application. It was discovered that the viscosity of the Acyclovir-Loaded Transferosomal Gel was 4452 cps.

Spreadability

It is a crucial component of patient compliance since gels that spread uniformly and readily are typically favored. A gel with good spreadability will cover the targeted area evenly, which can improve the drug's effectiveness. It was discovered that the prepared Transferosomal gel had a spreadability of 19.03 g.cm/sec. This suggests that when the gel is put to skin, it can spread out with ease.

Drug content

The gel's ability to effectively treat the intended condition is ensured by its accurate drug content. Variability in the composition of drugs can impact both safety and efficacy and result in uneven therapeutic outcomes. It was found that 98.03% of acyclovir in transferosomal gel was present.

In-vitro drug Release:

F5 improved by 1%. Carbopol-940 gel had the most drug release (58.35 percent for 12 hours), the best homogeneity, the maximum drug concentration, and the appropriate viscosity. It was therefore regarded as an optimum formulation. Higher concentrations of soy lecithin and Tween 80 in transferosomes lead to enhanced drug release (F5). This might be due to increased fluidity and flexibility. Both soy lecithin and Tween 80 increase the fluidity and flexibility of the bilayer, facilitating easier drug release.

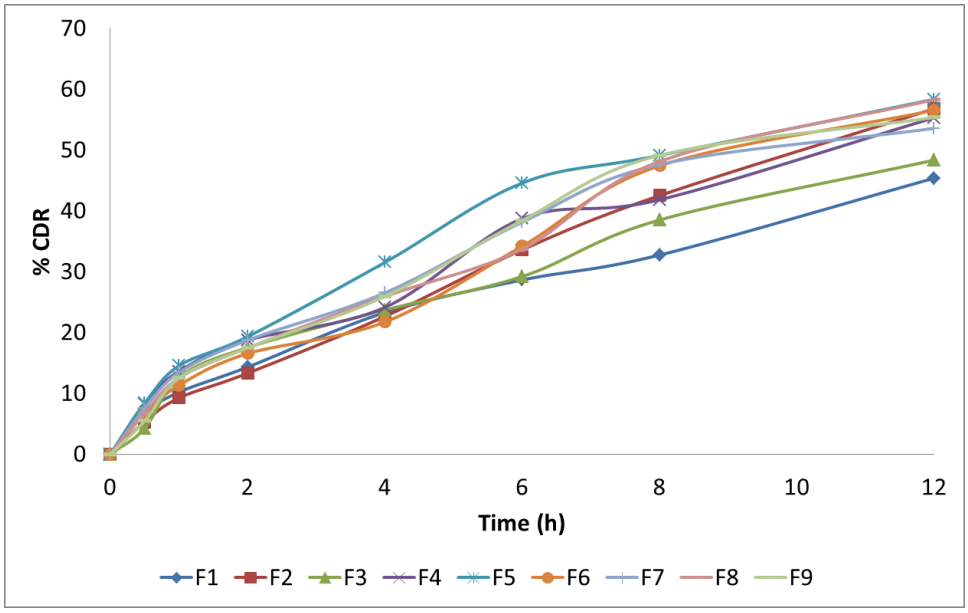

Figure 7: *In-vitro* drug release study

Drug release kinetics

The *in vitro* release data were inserted in the Zero order, First order, and Higuchi equations to find the release model that best reflects the pattern of drug release. The system where the drug release rate is independent of its concentration is described by the zero order rate. Drug release over time is plotted against cumulative amount using zero order kinetics. The drug release rate is dependent on its concentration, as indicated by the first-order rate. Based on Fickian diffusion, the Higuchi model explains drug release from an insoluble matrix as a square root of a time-dependent process.

The regression coefficient, or r^2, and the release constant were computed based on the slope of the relevant plots. The findings are shown in Table 7. The mechanism of drug release from the transferosomal gel was found to be diffusion controlled because plots of percent cumulative drug release versus square root of time were found to be linear with the regression coefficient, r^2 values ranging from 0.8762 to 0.9832 for all the formulations. The kinetics of drug release from the transferosomal gel was found to follow first order after the comparative evaluation of r^2 values. The plots of log cumulative percentage drug release versus time were linear (r^2 value ranged from 0.8176 to 0.9412).

Table 7: Analysis of kinetic release model of formulations

Formulation Code	Regression Coefficient (R^2)		
	Zero Order	First Order	Higuchi Model
F1	0.7962	0.8933	0.9218
F2	0.8132	0.8613	0.9768
F3	0.7936	0.8176	0.8867
F4	0.8515	0.8927	0.8762
F5	0.9021	0.9412	0.9832
F6	0.8135	0.8253	0.8912
F7	0.8942	0.8846	0.9135
F8	0.8253	0.9132	0.9553
F9	0.8726	0.8315	0.9374

CONCLUSION

The acyclovir-loaded transferosomal gel's composition has been successful in producing the qualities needed for efficient transdermal drug delivery. The drug content of the gel is in compliance with therapeutic criteria, and its pH, viscosity, and spreadability are all within ideal levels for user comfort and practical application. These results imply that for situations needing topical antiviral therapy, the transferosomal gel may offer an efficient and practical way to administer acyclovir. The gel's potential as a competitive substitute for conventional topical formulations is highlighted by its capacity to preserve drug stability, improve skin penetration, and provide ease of use.

Acknowledgement: Authors are thankful to the Department of Pharmaceutics, Shree Santkrupa College of Pharmacy, Ghogaon for providing necessary facilities for this work.

Author's Contribution: All authors equally contribute to this work.

Conflicts of Interests: None

Ethical approval: Not applicable

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