Formulation and Evaluation of Niosomal Gel containing Abacavir Sulfate

Running Title: Abacavir sulfate niosomal gel

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ABSTRACT:

Background: The present work was planned with a view to prepare niosomes of an Abacavir sulfate (antiviral drug) and incorporate them into suitable gel base to improve the residance time of the drug in skin layers, while decreasing the systemic absorption of drug..

Hypothesis: The niosomal drug delivery of abacavir sulfate will improve drug permeation.

Methods: Film hydration method was used to prepare the niosomes.. 3^2 Factorial design was used to optimized the niosomal formulation with two independent variables of span 60 and cholesterol amount. The properties like microscopic size, entrapment efficiency, and zeta potential were evaluated for the niosomes. The data was statistical analysed using DOE. The optimized batch of niosomal formulation was incorporated in gel base for topical application. The niosomal gel was tested for controlled drug release from the niosomes.

Results: The stable multilamellar vesicles (0.24 to 9.4 µm) were obtained. Variables such as amount of surfactant and cholesterol were found to have a significant impact on the vesicle size and percent entrapment effectiveness. The niosomes were found to be more stable at refrigerator temperature than the room temperature. The formulations were optimized at pH slightly less than 7. The spreadability and viscosity of the gel formulations showed an inverse correlation. Niosome formulations outperformed conventional formulations in ex-vivo experiments on percutaneous permeation, as measured by skin penetration and drug deposition.

Conclusion: According to the information provided by this study, niosomal vesicles are a useful carrier to improve the penetration and deposition of the Abacavir sulphate in the skin,

KEYWORDS: Abacavir sulfate, antiviral drug, niosomes, multilamellar

1. INTRODUCTION

It has been discovered that colloidal lipid-based vesicular drug delivery methods like liposomes and niosomes are preferable to traditional dosage forms. These systems serve as drug reservoirs and release the medication in a controlled manner. Additionally, altering their content, composition, or surface characteristics can help with action location and targeting. Niosomes, which are non-ionic surfactant-based vesicles, were created as an alternative to liposomes for regulated drug delivery because the latter require sterilising and are unstable in large-scale manufacture (1). These hydrated mixes of cholesterol and non-ionic surfactants, such as alkyl ether, alkyl ester, or alkyl amide, form the tiny lamellar structures (2). The mechanism of vesicle formation when using nonionic surfactants is not fully understood. Basically, these vesicles do not form spontaneously, but due to their amphipathic properties they form closed bilayers of nonionic surfactants in aqueous media. Some energy is used during the formation of niosomal structures such as physical agitation and heat (2). These vesicles are often formed as unilamellar structures or multilamellar structures in structure depending upon the tactic of preparation.

In this closed bilayer structure, the hydrophilic tails are exposed on the surface and within the vesicle, and the hydrophobic chains face each other within the bilayer. Thus, vesicles retain hydrophilic drugs in the space enclosed by the vesicles, whereas hydrophobic drugs are embedded in the surfactant bilayer itself (2). In niosomes, vesicles are formed from surfactants such as Span-60. Span-60 is nonionic in nature and is stabilized by the addition of small amounts of anionic surfactants such as cholesterol and diacetyl phosphate. (3). Niosomes are promising vehicles for drug delivery, are nonionic, have low toxicity, and have improved the therapeutic index of drugs by limiting their effects to cellular concentrations.

Abacavir sulfate

Abacavir sulfate is chemically a nucleoside polymerase inhibitor with antiretroviral activity against HIV. The potential of niosomes is now becoming more realized to medical field because they

overcome toxicological and immunological difficulties related to the drug. Niosomes are vesicular drug delivery systems offering advantages in topical route.

The present study was to analyze the feasibility of using niosomes as a topical drug delivery system for selected medicinal drug. The standard dosage variety of antiviral agent has several side effects like nausea, vomiting and abnormalities of liver enzymes. In present work efforts were made to organize encapsulated niosomes of Abacavir sulfate (antiviral drug) and incorporated into suitable gel base which were further evaluated for its controlled drug delivery (4). The present work will be beneficial to improve drug permeation.

2. MATERIALS AND METHODS

2.1. Materials:

Abacavir sulfate was procured from Micro lab, India. The surfactants, cholesterol was purchased from SRL Chemicals and Span 60 was purchased from Samar Chemicals. Other required chemicals Potassium dihydrogen ortho phosphate, Chloroform, and Methanol were procured from S. D. Fine Chemicals. Isopropyl alcohol was purchased from SRL Chemicals, India. Dialysis membrane was obtained from HI Media Laboratories, Membrane filters were obtained from Pall Corporation, India.

2.2. Methods

2.2.1. Preparation of standard calibration curve of Abacavir sulfate in pH 6.8 phosphate buffer.

A standard calibration curve for abacavir sulfate was generated over the concentration range 5-50 (µg/mL) using pH 6.8 phosphate buffer (5).

2.2.2. Drug-excipients interaction study

The physical mixture of drug, cholesterol and surfactant as potassium bromide dispersion was subjected to FT-IR spectroscopic analysis. The comparison of the FT-IR spectra of drugs and physical mixtures was done (6).

2.2.3. Preparation of Abacavir sulfate loaded niosomes by film hydration method

Surfactant span-60 and cholesterol were weighed precisely and dissolved in 10 ml of the chloroform: methanol combination (2:1, v/v) along with 50 mg of Abacavir sulphate (6). In order to produce a thin film on the flask wall and remove all organic solvents, the solvents were extracted under vacuum in a rotary evaporator at 50°C for 1 hour. The solvents were then held under vacuum for 2 hours. Using 10 ml of pH 7.4 phosphate buffer, which is above the gel-liquid transition temperature (Tc) of sorbitan monoesters, for one hour at 60°C, the surfactant film was hydrated. Using the horizontal mechanical shaking water bath, the resulting suspension was mechanically shook for one hour. The dispersion was sonicated three times for a total of one minute on and one minute off to create multilamellar niosomes after being left at room temperature for four hours to fully hydrate. (7).

2.2.4 Designing of factors for niosomal formulations

The designing of the formulation with the assistance of the factorial design assists in optimization purpose of niosomal formulations containing different amounts of span and cholesterol. A method of experimental design known as factorial design allows for the evaluation of the components that are involved and their significance to the study. The runs or formulations used in the current investigation were created using a 3^2-factorial design with two factors (Lipid and Surfactant Concentration) tested at three levels, and experimental trials were carried out using every feasible combination. **Table No. 1 and Table No. 2** (8).

Table No. 1: Factorıal desıgn layout

Factors (independent variables)	**Levels used**			**Responses (dependent variables)**
	-1	0	+1	
A = amount of span 60(mg)	40	50	60	R1 = % entrapment
B = amount of cholesterol(mg)	20	30	40	R2 = mean vesicle size(nm)

Table No. 2: Formula of factorıal batches

Ingredients	**Batches**								
	N1	N2	N3	N4	N5	N6	N7	N8	N9

Abacavir Sulfate (mg)	50	50	50	50	50	50	50	50	50
Span 60 (mg)	40	40	40	50	50	50	60	60	60
Cholesterol (mg)	20	30	40	20	30	40	20	30	40

2.2.5. Optımızatıon

The runs that were created using 3^2 full factorial designs had their response variables assessed. Multiple regression analysis was performed on the response values to determine the link between the influencing factors and the resulting response values.

For the following relationship, the mean vesicle size and % entrapment efficiency were chosen.:

1. The impact of span 60 on vesicle size and entrapment effectiveness
2. The impact of cholesterol concentration on vesicle size and entrapment effectiveness.

2.2.6. Statistical analysis

Using the commercially available software programme Design of Experiments® 8.0.7.1, one-way ANOVA was applied at the 0.05 level to statistically examine the impact of formulation factors on the answers (Stat Ease, USA)(8).

2.2.7. Evaluation of Abacavir sulfate loaded niosomes

2.2.7.1.*Entrapment efficiency*

The effectiveness of the drug's encapsulation within niosomes was assessed using a dialysis procedure to remove the drug from the niosomes. A dialysis bag was filled with 2 ml of a drug-loaded niosomal dispersion, which was then rigorously dialyzed in 200ml of phosphate buffer pH 6.8 while being swirled magnetically at 150 rpm and 370.5°C for five hours. After being broken up with 4 ml of isopropyl alcohol, the vesicles were diluted with pH 6.8 phosphate buffer up to 10 ml, and then sonicated for 15 min. With pH 6.8 phosphate buffer, 1 ml of the resulting dispersion was diluted up to 25 ml, and the drug content was determined using UV spectroscopic analysis. (7). The % entrapment efficiency was determined by using following equation.

$$\%EE=[(Ct-Cf)/Ct]\ 100$$

Where, Ct – Total drug concentration

Cf- Free drug

2.2.7.2.*Measurement of particle size and zeta potential*

The average particle size and size distribution were determined using the Malvern Zetasizer Nano (polydispersity index, PI). The photon correlation spectroscopy (PCS) approach served as the foundation for this equipment. The light source was a monochromatic coherent He-Ne laser with a set wavelength of 633 nm. Measurements were made of the statistical intensity variations in the light reflected from the particles. These variations were brought on by the particles' unpredictable Brownian motion. The Malvern Zetasizer Nano was used to prepare the appropriate dilutions for determining zeta potential. Each sample was run three times, with analysis done at 25 °C and a detection angle of 173 ° for both particle size and zeta potential.(9).

2.2.8. Preparation of niosomal gels

To prepare an aqueous dispersion, carbopol-934 was dissolved in a tiny amount of distilled water. The dispersion was stirred and left to hydrate overnight. Niosomal suspension in the desired amount (100 mg of medication) was added while being gently stirred. The amount of propyl and methyl parabens were dissolved in a little amount of water and added to the dispersion. Distilled water optimised the gel's ultimate weight. Triethanolamine was added to the gel to bring the pH level down to 6.8–6.7. To release the trapped air, the gel was allowed to stand overnight under vacuum. (7).

2.2.9. Evaluation of niosomal gels

2.2.9.1 Appearance

Visual examination of the prepared gels was done to check for particle presence, colour, and clarity.

2.2.9.2. pH measurement

The pH was measured after the pH meter had been calibrated and 1 g of gel had been dissolved in 20 ml of distilled water (7).

2.2.9.3. Viscosity

All gel samples' viscosities were measured using a Brookfield viscometer. (7).

2.2.9.4. Spreadability

By placing 0.5 g of each gel in a circle 1 cm in diameter that was premarked on a glass plate over which a second glass plate was placed, the spreadability of niosomal gel and gel base was tested. For around 15 seconds, a weight of 500 g was permitted to rest on the upper glass plate. The spread of the gel was measured in diameter (7).

2.2.9.5. Drug content

100 mg of the vesicular gel sample was dissolved in 10 ml of methanol after it had been sufficiently diluted. By utilising a UV spectrophotometer to detect the absorbance at a maximum wavelength of 250 nm, the drug content was computed using a reference graph. (7).

2.2.9.6. In-Vitro Release Study

For the in-vitro diffusion investigation utilizing cellophane membrane, a modified Franz diffusion cell with a 15 ml capacity was used. By soaking the cellophane membrane overnight in a solution of ethanol and phosphate buffer 7.4, the membrane was activated. The receiver compartment received a segment of membrane that had been cut, measured, and applied. Niosomal formulation was placed into the donor compartment. As the receptor medium, a 15 ml aliquot of a 4:6 (v/v) ethanol: pH 7.4 phosphate buffer was used. A magnetic bar was used to stir the receptor media at 300 rpm while keeping the temperature at 37°C. The receptor media was taken out in aliquots of 0.5 ml every hour for up to 12 hours. Using receptor media, the 0.5 ml sample was appropriately diluted before being spectrophotometrically examined at For the in-vitro diffusion investigation utilising cellophane membrane, a modified Franz diffusion cell with a 15 ml capacity was used. By soaking the cellophane membrane overnight in a solution of ethanol and phosphate buffer 7.4, the membrane was made active. The receiver compartment received a segment of membrane that had been cut, measured, and applied. Niosomal formulation was placed into the donor compartment. As the receptor medium, a 15 ml aliquot of a 4:6 (v/v) ethanol: pH 7.4 phosphate buffer was utilised. A

magnetic bar was used to stir the receptor media at 300 rpm while keeping the temperature at 370C. The receptor media was taken out in aliquots of 0.5 ml every hour for up to 12 hours. Utilizing receptor media, the 0.5 ml sample was appropriately diluted before being spectrophotometrically examined at λmax 250 nm (10).

2.2.9.7. Ex-vivo permeation study

Diffusion investigations were conducted using the Franz diffusion cell, which has an effective diffusion area of 3.14 cm2. The dermal side of the Sprague Dawley rat abdominal skin sample, which had been dehaired and defatted, was put above the diffusion cell in contact with the receptor phase. Diffusion medium (phosphate buffer solution, pH 7.4) was added to the receptor compartment, which had an effective capacity of 20 ml, and stirred at 150 rpm with a glass bead using a magnetic stirrer. A water bath was used to equilibrate the test system at 37 0.5 °C. 300 mg of gel was applied to the skin and evenly distributed over a 3.14 cm2 test area. At certain intervals, the receptor compartment's contents were taken out and replaced with new medium to undertake serial sampling of the dermal compartment. Each trial was carried out for 24 hours, during which time it was calculated how much of the drug was delivered onto the rat's skin. (11).

2.2.9.8. Treatment of drug release data with different kinetic equations

The optimal mathematical model to use is the one that best fits the results of the experiments when explaining the kinetics of the drug release process. The best match of the dissolving data to various models was found to calculate the kinetics of Abacavir sulphate (12).

2.2.9.10. Determination of the drug content in skin

The extra vesicular gel was eliminated, and the skin samples were then gently dried with cotton wool after being rinsed with alcohol and water to remove any remaining Abacavir sulphate from the surface. After that, the dry skin was placed in 10 ml of methanol and sonicated for 45 minutes. The solution was run through a 0.45 m-pore membrane filter before being subjected to UV spectroscopy at 250 nm for analysis (13,14).

3. STABILITY STUDY

The stability studies of optimized niosomal suspension (ON10-C) were carried out at referigerator temperatuere (4-8°C) and at room temperature for 60 days (9).

4. RESULTS

4.1. Drug-excipients interaction study

All excipients were found to be compatible with Abacavir sulfate, as all the characteristic peaks (cm^{-1}) of Abacavir sulfate were observed within the FT-IR spectrum of the physical mixtures of drug and excipients. **Fig 1**

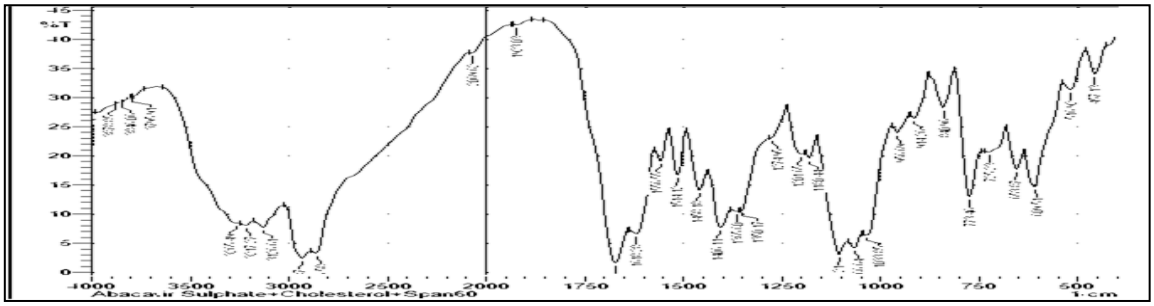

Fig 1: FTIR spectrum of drug, span 60 and cholesterol

The characteristic IR absorption peaks of Abacavir sulfate**,** cholesterol and of span 60 at different frequency ranges were obtained. The IR spectrums of Abacavir sulfate, cholesterol, span60 and their physical mixture were found to be identical**.** The interpretation of FT-IR graphs spectra obtained indicated that no interaction occurred between the Abacavir sulfate and other ingredients used in formulation. The physical mixture's FT-IR spectra showed absorption peaks resembling those in the separate spectra of the pure drug sample and components.

4.2. Evaluation of Abacavir sulfate loaded niosomes:

4.2.1. Characterization of Abacavir sulfate niosomes

The Abacavir sulphate niosomes were characterized for percent entrapment efficiency, zeta potential and mean vesicle size. The results are mentioned in **Table No. 3**

Table No. 3: Percent entrapment efficiency, zeta potential vesicle size, of niosomal formulations

Sr. No.	Formulation	Mean EE (%) ± S.D.	Z-Avg diameter (mean+SD)(nm)	PDI (mean+SD)	Zeta potential (mean+SD)(Mv)
1	N1	63.74±0.05	192±0.84	0.325±0.29	-27.77±1.55
2	N2	77.88±0.01	194±0.86	0.289±0.23	-24.84±0.79
3	N3	82.10±0.06	*199±0.97*	0.341±0.09	-23.35±1.87
4	N4	80.25±0.22	211±0.90	0.285±0.05	-25.44±1.86
5	N5	75.09±0.06	215±0.77	0.225±0.02	-28.07±0.75
6	N6	74.04±0.03	219±0.80	0.207±0.12	-31.57±0.16
7	N7	84.80±0.02	182±0.59	0.267±0.31	-29.69±1.03
8	N8	66.32±0.04	186±0.61	0.304±0.04	-28.27±0.28
9	N9	70.56±0.1	189±0.55	0.259±0.038	-30.55±0.28

* All values are expressed as mean ± SD (n=3)

As shown in the niosomal photomicrographs,the shape was found to be perfect sphere in the size range of 0.24 to 9.4 μm due to formation of multilamellar vescicles with no aggregation. The zeta potential values predicts the stability of nisomal formulations.

From a pharmacological perspective, the most crucial factor in niosomal formulations is the entrapment efficiency. Niosome entrapment efficiency increases with the use of cholesterol up to a point. The considerable decrease in drug entrapment on increase in cholesterol content was observed.

The bilayer hydrophobicity and stability rise and the permeability decreases with the required amount of cholesterol, which may effectively trap hydrophobic drugs into the bilayers as the vesicles form. Contrarily, a drug's quantity may compete with cholesterol, preventing it from being a part of the amphiphilic structure. It was discovered that the span 60 had a much higher trapping efficiency. This could be due to chemical composition and highest phase transition temperature.

4.2.2. Optimization

The response variables are assessed for the runs or formulations, which are created using 3^2 full factorial designs. Multiple regression analysis is applied to the response values in order to ascertain the relationship between the factors and the response values. For this study's analysis, the response values are:

1. Percent Entrapment efficiency
2. Mean vesicle size

4.2.3. Optimized formulation

Thus, for a given response parameter, the polynomial equations were used to derive the optimum formulations. Numerical optimization in Design Expert 8.0 Version was used to arrive at the optimum formulas during the trial runs. The 3^2 full factorial designs were used to create the niosomal formulations. In this study, the impact of formulation variables were chosen as independent variables, including the quantity of cholesterol and span 60. The mean vesicle size and % entrapment effectiveness are among the dependent (response) variables. Only significant (p 0.05) coefficients were used to create polynomial models.

The results of multiple linear regression analysis showed that a decrease in the percentage of release is seen as cholesterol and span 60 concentrations rise above a certain level.

4.2.4. Statistical analysis

Values of "Prob > F" less than 0.0500 indicate model terms are significant. During this case A, B, A2 are significant model terms. Values greater than 0.1000 indicate the model terms aren’t significant**.**

For entrapment efficiency, the Model F-value of 211.05 implies the model is critical.

For vesicle size, the Model F-value of 482.10 implies the model is critical.

The R square value and adjusted R square value are given in **Table No. 4** for both the factors.

Table No. 4: Anova for selected factorıal model (entrapment effıcıency and vesıcle sıze)

Parameter	Source	Sum of	Df	Mean	F	p-value	
Entrapment Efficıency	Model	399.40	2	199.70	211.0	<0.	Significant
	A-Span 60	360.84	1	360.84	381.3	<0.	
	B-	38.56	1	38.56	40.75	0.0	
	Residual	5.68	6	0.95			
	Cor Total	405.078	8				
Vesicle Sıze	Model	1428.44	5	285.69	482.1	0.0	Significant
	A-Span 60	130.65	1	130.67	220.5	0.0	
	B-	8.67	1	80.67	136.1	0.0	
	AB	0.00	1	0.000	0.000	1.0	
	A2	1216.89	1	1216.8	2053.	<0.	
	B2	0.22	1	0.22	0.37	0.5	
	Residual	1.78	3	0.59			
	Cor Total	1430.22	8				

4.2.5. Search for Optimized Formulation

The value of entrapment efficiency and vesicle size served as the primary criteria for choosing an appropriate viable location. The following criteria were used to choose one formulation:

Region: Vesicle size is 182-219 nm, % Entrapment Efficiency is 63.74–84.80%

Based on the outcomes of thorough grid searches, an integrated search for formulation batch N1–N9 was carried out. The design expert software's solution was applied to optimise the vesicle size (nm) and% Entrapment efficiency. The generated formulations in four distinct batches were examined for the evaluation of the chosen parameters, namely vesicle size (nm) and% Entrapment efficiency. The result are as given in **Table No. 5, Fig. 2**

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Table No. 5: Solution of % entrapment efficiency and vesicle size for optimization

Number	Span 60	Cholesterol	%Entrapment Efficiency	Vesicle size(nm)	Desirability
1	0.00	-1	77.511	211.444	1

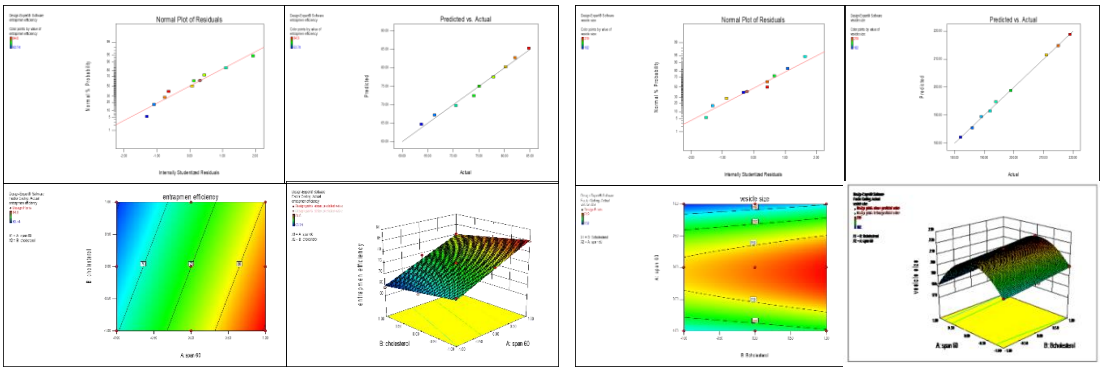

Fig 2: A- Normal plot of residuals, B- Plot of predicted vs actual response, C- Effect of factors, D- Response surface plot of % entrapment efficiency, E- Normal plot of residuals, F- Plot of predicted vs actual response, G- Effect of factors, H- Response surface plot of Vesicle Size

Stability Study

It was observed that ON10-C and ON10-D niosomal suspensions had higher stability in terms of entrapment efficiency over the other niosomal suspensions. On10-A-containing niosomal suspension was shown to have poor stability. This is thought to be caused by the system's low zeta potential, which results in little electrostatic repulsion. Their increased vesicle size and polydispersity index may also have had a role in this system's decreased stability. **Fig 3**

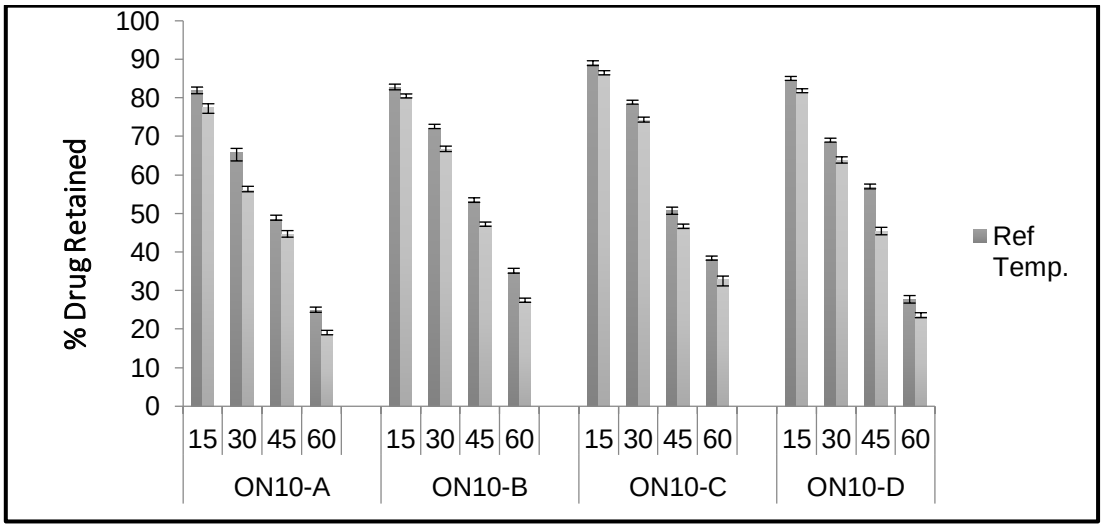

Fig 3: Effect of storage time and temperature on the entrapment efficiency of vesicles

It was found that the low temperature does not shoe much effect on percent entrapment efficiency. Thus the niosomal Batch ON10-C was found to be more stable at low temperature and can be stored at this temperature.

4.2.6. Evaluation of niosomal gels

The % drug content, pH, spreadability, and viscosity of the improved batches of niosomal gels as well as traditional gel were assessed. The results are mentioned in **Table No. 6 and Table No. 7** The prepared vesicular gels were found to be clear with shining milky appearance.

Table No. 6: Percent entrapment efficiency and mean vesicle size (nm) for optimized formulations (ON10-A TO ON10-D)

Sr. No.	Batch code	Mean Particle size (nm) ± S.D.	% Entrapment Efficiency
1	ON10-A	199 ± 0.97	75.98 ±0.12
2	ON10-B	225± 0.06	76.38± 0.08
3	ON10-C	215 ± 0.77	77.46 ±0.41
4	ON10-D	268 ± 0.12	78.59± 0.01

* All values are expressed as mean ± SD (n=3)

4.2.7. pH measurement

The pH of the human skin lies in between 4.5 to 6, the pKa of the Abacavir sulfate is 7.1 and carbopol gives good viscosity in the pH range of 6 to 11. By considering all of these factors, formulations' pH levels were tuned to be slightly below 7 to be appropriate for topical administration..

4.2.8. Viscosity

The formulations' viscosities were found to have a linear correlation with the concentration of the respective niosomal suspension.

4.2.9. Spreadability

The optimized formulations were found to have adequate spreadability which indicates the ease of application. A large diameter of spreading indicates better spreadability. The spreadability was found to have an inverse correlation with the viscosity of the formulation.

4.2.10. Drug content

The drug content for the gel-infused, optimised batch was identified.. The batch ON10-C showed maximum drug content in percent.

Table No. 7: Values of dıfferent evaluatıon parameters of vesıcular gel

Sr. No.	Batches	Percent Drug content (mean+SD)	pH (mean+SD)	Spreadability (cm) (mean+SD)	Viscosity (cPs) (mean+SD)
1	ON10-A	94.8±0.821	6.90	3.966±0.057	5600±24.4
2	ON10-B	98.7±0.611	6.92	4.333±0.057	4800±22.0
3	ON10-C	99.3±0.832	6.88	3.33±0.057	6400±25.3
4	ON10-D	94.9±1.222	6.79	3.23±0.057	9200±28.5
5	Conventional gel	100 ±0.401	6.83	3.666±0.057	4400±21.4

*All values are expressed as mean ± SD (n=3)

4.2.11. In-Vitro Release Study

Modified Using a cellophane membrane, an in-vitro diffusion research was conducted using a 15 ml Franz diffusion cell. The results of drug release from the gels are as given in the **Fig 4**

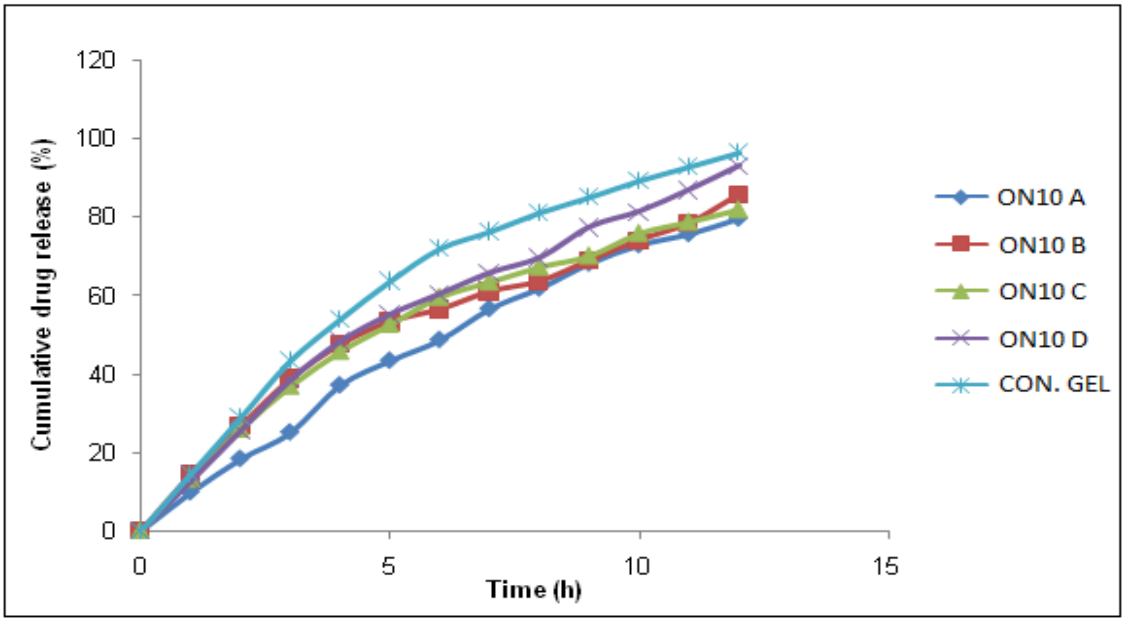

Fig 4: Cumulative percentage *In Vitro* drug release from formulations ON10-A to ON10-D with conventional gel

4.2.12. Ex-vivo permeation study

Diffusion investigations were conducted using the Franz diffusion cell, which has an effective diffusion area of 3.14 cm^2. Niosomal gel formulations was applied to rat skin that had been defatted and dehaired allowed researchers to determine the cumulative percentage drug release. The results are shown in the **Fig. 5**

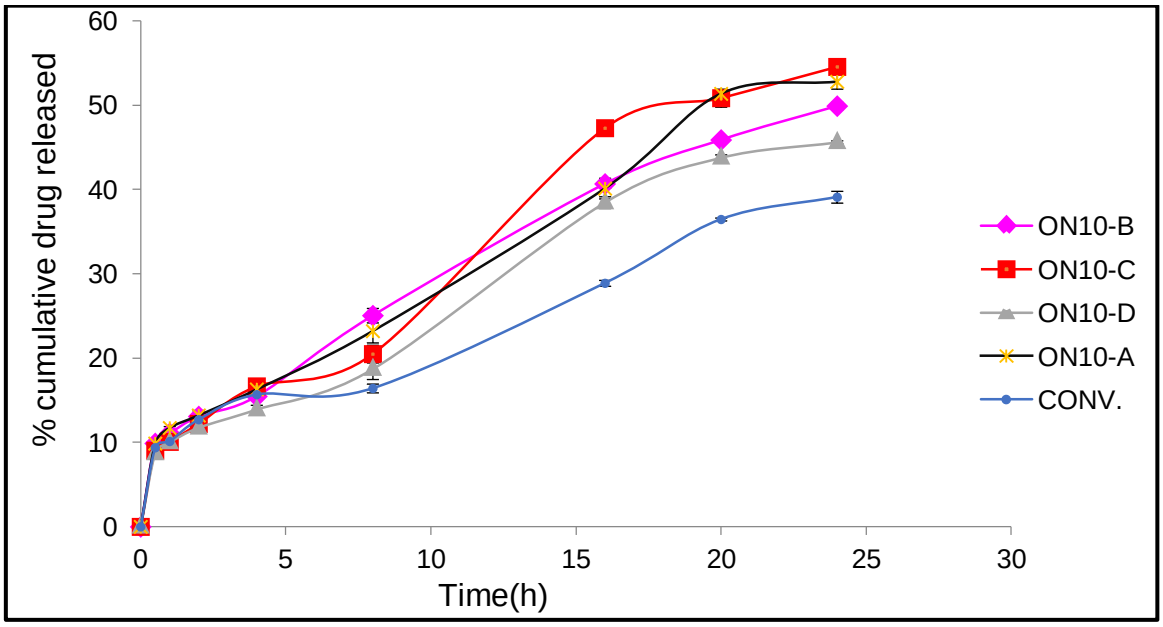

Figure 5: Cumulative percentage *Ex-vivo* drug release from formulations ON10-A to ON10-D

In comparison to the standard gel, the niosomal gels showed better ex-vivo penetration and skin partitioning of the medication. Abacavir sulphate penetration from the niosomes may have been improved in part by the surfactant's role as a permeation enhancer in the vesiculr formulations. Another theory was that the stratum corneum's niosomes merged at the interface, and the stratum corneum's upper layer was very active with Abacavir sulphate due to the high local drug concentration in the bilayers.

4.2.13. Treatment of drug release data with different kinetic equations:

Different kinetic models were applied to the drug release results that were obtained in vitro. Finding the best fit of the dissolving data to various models—Zero order, first order, Higuchi, and Peppas—led to the identification of the kinetics of abacavir sulphate. The results are given in table **Table No. 8 and Fig 6, 7, 8.**

Table No. 8: Kinetic treatment of drug release data of various formulations ON10-A to ON10-D

Formulation code	Zero order	First order	Higuchi's matrix	Peppas model
	R^2			
ON10-A	0.9704	0.7534	0.9677	0.9325
ON10-B	0.9657	0.7763	0.9834	0.9491
ON10-C	0.9662	0.6594	0.9571	0.9455
ON10-D	0.9647	0.8757	0.9611	0.9272

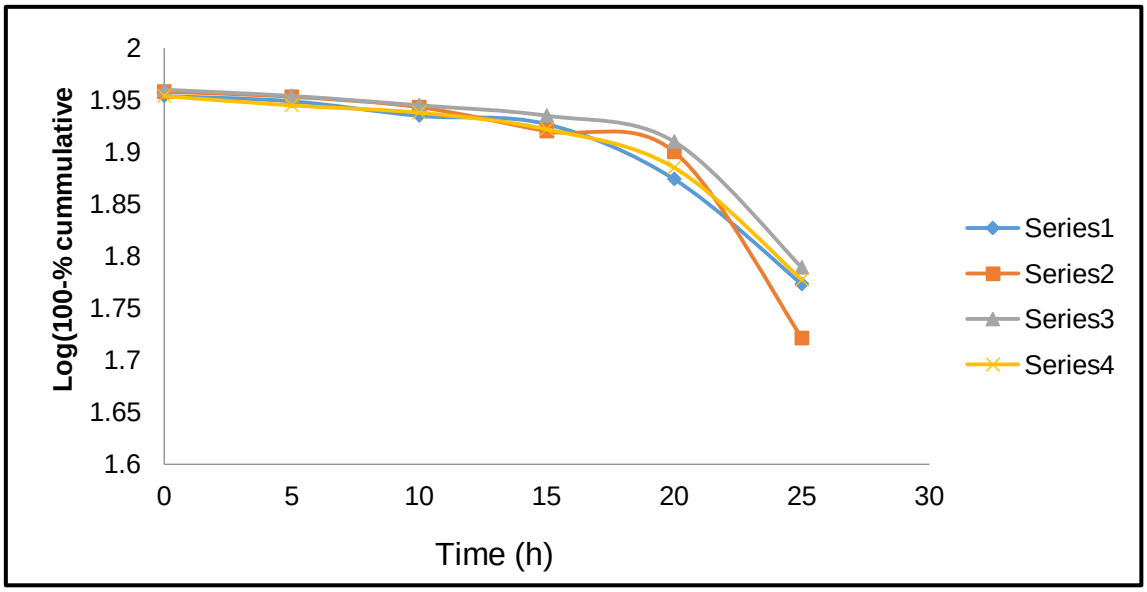

Figure 6: First order plot of drug release of formulations ON10-A to ON10-D

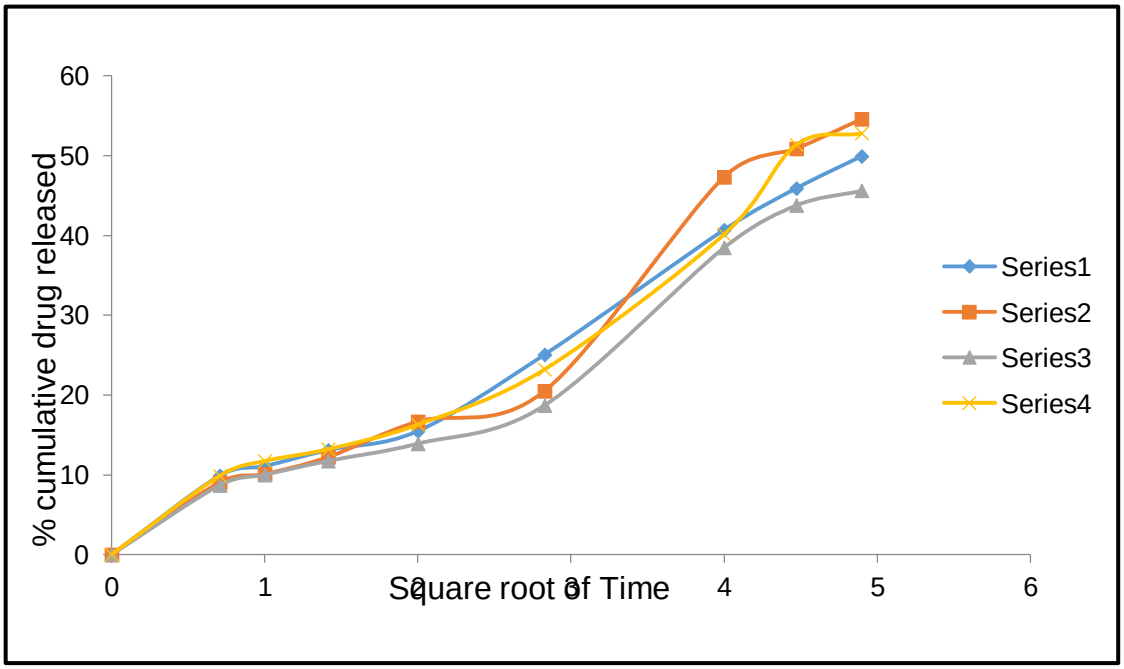

Figure 7: Higuchi´s plot of drug release of formulations ON10A to ON10D

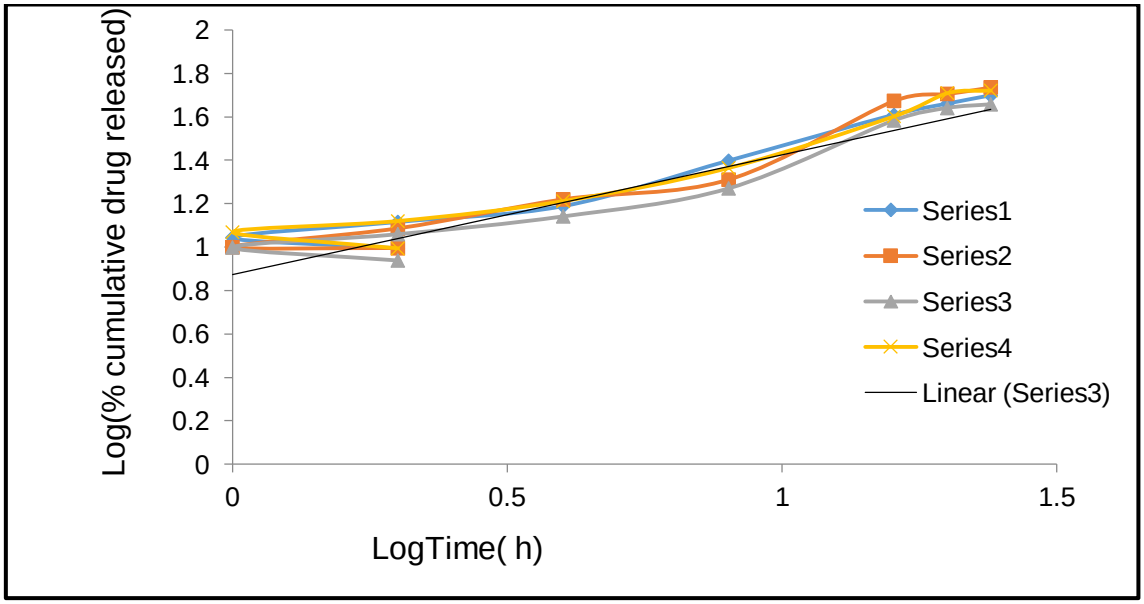

Figure 8: Korsmeyer peppas plot of drug release of formulations ON10-A to ON10-D

Niosome composition and gelling polymer viscosity both affect the mechanism and kinetics of drug release. To determine the mechanism of drug release, the in-vitro drug permeation data was subjected to a goodness of fit test using linear regression analysis in accordance with zero order, first order kinetic equations, Higuchi, and Korsmeyer models.

Drug release from all formulations was determined to follow zero order kinetics when the regression coefficient "R2" values of zero order and first order plots were compared. These values ranged from 0.9647 to 0.9704.

4.2.14. Determination of the drug content in skin

The percentage of Abacavir sulphate absorbed inside the skin layers increased greater with the niosomal formulations than with the conventional formulation, it is important to note. It was found that the niosomal and conventional formulations have a clear difference in the net amount of medication deposited on skin. The previous formulation had very little capacity to penetrate the skin. The results are given in the **fig 9**

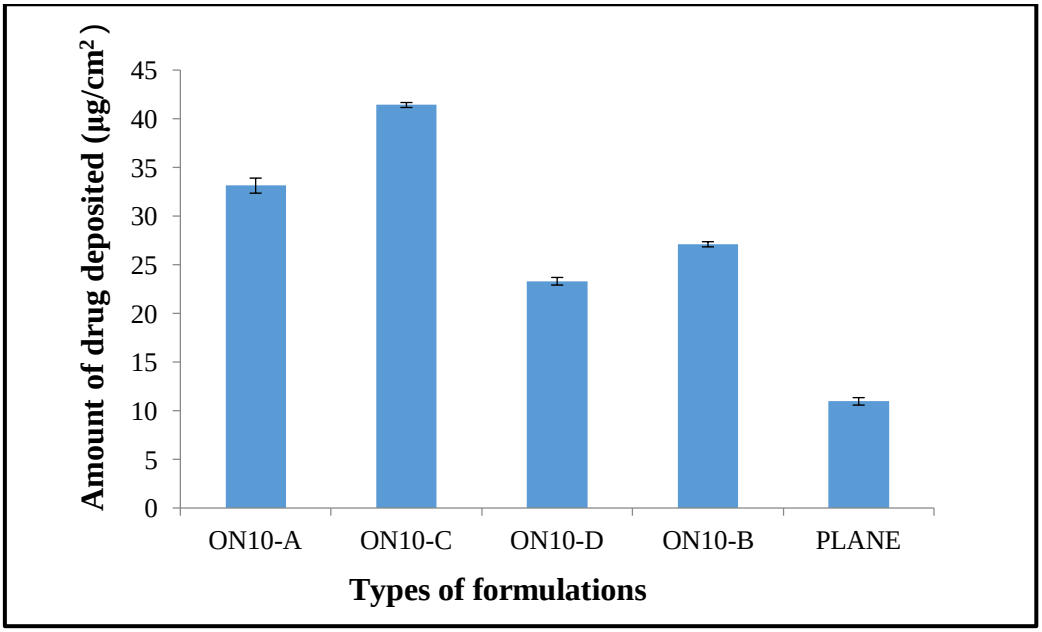

Figure 9: Drug deposition in rat skin from different formulations, (Mean±S.D) (n=3)

The formulation ON10-C showed the highest skin absorption of the drug; this may be because the formulation's medium viscosity and desirable vesicle size resulted in a greater drug release from the vesicles. The skin accumulation rate for formulations with a medium viscosity and small vesicle size should be noticeably greater. The very high viscosity (9200 cps) of the formulation, which

decreased the drug diffusion coefficient, may be the cause of the poor skin accumulation of abacavir sulphate from the formulation ON10-D.

4.2.15. Drug accumulation in the skin

The maximum accumulation of the drug in the skin was observed in ON10-C, owing to better drug release from niosomes and medium viscosity of formulation as compared to other batches. The enhanced penetration effect of the surfactant and the smaller particle size of niosomes could be attributed to enhanced bioavalibility of Abacavir sulfate within the skin.

The results obtained from the permeation study followed by the Abacavir sulfate skin content study clearly implicit that the niosomes were able to greatly improve the cutaneous retention of drug. In conclusion, the results obtained during these *ex-vivo* permeation studies have shown that the niosomes are better carriers than the conventional formulation.

5. CONCLUSION

The formulation of Abacavir sulfate loaded niosomes was done by the film hydration method as it was found to be well-suited and sound approach at laboratory scale to obtain the stable multilamellar vesicles. The batces of niosomes were optimized using Design expert software. The excipients used in niosomel formulation were found to be compatible with the drug. Niosomes were found to be spherical multilamellar vesicles in the size range of 0.24 to 9.4 µm. Variables such as amount of surfactant and cholesterol were found to have a profound effect on the percentage entrapment efficiency and vesicle size. The niosomes were found to be more stable at refrigerator temperature than the room temperature. The formulations were optimized at pH slightly less than 7.

The spreadability and viscosity of the gel formulations showed an inverse correlation. In the *ex-vivo* percutaneous permeation studies, the niosomal formulations were found to be superior in skin penetration and drug deposition as compared to the conventional topical formulations. The formulation ON 10-C was found superior in terms of all the evaluation parameters along with the perscent drug release, drug penetration and drug deposition. This study proved that the niosomal

vesicles are beneficial as the topical drug delivery carrier to improve the penetration and delivery of the Abacavir sulfate in the skin.

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