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Research Article

# FORMULATION AND EVALUATION OF ACYCLOVIR LOADED NOVEL NANO-EMULSION GEL FOR TOPICAL TREATMENT OF HERPES SIMPLEX VIRAL INFECTIONS

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### ABSTRACT

Acyclovir has low bioavailability mainly due to low solubility. This study aimed to formulate an optimized acyclovir (ACV) nanoemulsion gel for the slow, variable and incomplete oral drug absorption in patient suffering from herpes simplex viral infection. The dispersion solubility of acyclovir was studied in various oils, surfactants and co-surfactants and by constructing pseudo phase ternary diagram nanoemulsion area was identified. The optimized formulations of nanoemulsions were subjected to thermodynamic stability tests. After stability study, stable formulation was characterized for droplet size, pH determination, centrifugation, % drug content in nanoemulsion, Zeta Potential and Vesicle size measurement and than nanoemulsion gel were prepared and characterized for spreadability, measurement of viscosity, drug content, *In-vitro* diffusion, *in-vitro* release data. Span 40 was selected as surfactant, PEG 400 as co surfactant and castor oil as oil component based on solubility study. The *in vitro* drug release from acyclovir nanoemulsion gel was found to be considerably higher in comparison to that of the pure drug. The *in-vitro* diffusion of nanoemulsion gel was significantly good. Based on this study, it can be concluded the solubility and permeability of acyclovir can be increased by formulating into nanoemulsion gel.

**Keywords:** Acyclovir, Nanoemulsion, *In-vitro* diffusion, Zeta potential, Stability

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### INTRODUCTION

Acyclovir (ACV) is a guanosine antiviral drug and is one of the antiviral drugs most commonly used for treatment of herpes simplex virus infection, as well as varicella zoster (chickenpox) and herpes zoster (shingles). Topical application of ACV is limited by low transdermal penetration and poor solubility in water. Many strategies have been used to improve the therapeutic efficacy of ACV, including chemical modification, liposomes and nanoparticles<sup>1, 2</sup>. ACV is slightly soluble in water, with solubility ranging from 1.2 to 1.6 mg/mL at room temperature<sup>3, 4</sup> has relatively low oral bioavailability (10%–30%), has a short plasma half-life and is absorbed from the gastrointestinal tract

via passive diffusion and by transporters but its absorption is slow, variable and incomplete<sup>5</sup>.

The term nanoemulsion (NE) refers to a type of emulsion with a droplet size in its internal phase ranging from 5 nm to 200 nm and forms spontaneously with a transparent appearance. It is stabilized by a strong interfacial film of surfactant and cosurfactant/cosolvent molecules. Techniques such as microfluidization, high pressure homogenization<sup>6</sup>, emulsion inversion point<sup>7</sup> and sonication<sup>8</sup> have been utilized in preparation of NEs. NEs have been reported to successfully deliver a wide variety of therapeutic agents. Nanoemulsions are also referred to as miniemulsions, ultrafine emulsions and submicron emulsions. Phase behaviour studies have

shown that the size of the droplets is governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced by either temperature or composition. The capacity of nanoemulsions to dissolve large quantities of hydrophobics, along with their mutual compatibility and ability to protect the drugs from hydrolysis and enzymatic degradation<sup>9</sup>. The objective of this study was to develop and characterize nanoemulsion gel of acyclovir for increasing solubility and permeability across the biological membrane to improve the bioavailability, dosing frequency and enhance patient compliance.

## MATERIALS AND METHODS

Acyclovir was obtained as a gift sample from Macleods pharmaceuticals, Mumbai. Span 40, PEG 400 and castor oil were purchased from S. D. Fine Chem. Ltd., Mumbai. All other surfactant and co surfactant were purchased from Hi Media, Mumbai. Double distilled water was prepared freshly and used whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

### Solubility studies

Solubility determination in the various oils, surfactants and co-surfactants for formulating nanoemulsion drug delivery system. The solubility of the drug in different oils is an essential step for the nanoemulsion formulation. So before starting the phase diagram one must have to select the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is essential for the formulation of nanoemulsion drug delivery system. The solubility of ACV was determined by dissolving an excess amount of the drug in 2 ml of each liquid separately. The mixtures were shaken at 25°C±0.5°C for 48 hours in a water bath. After reaching equilibrium, the mixtures were centrifuged at 3,000 rpm for 15 minutes

using a centrifuge REMI laboratory, Mumbai. The supernatant was diluted with ethanol and the concentration of ACV was quantified spectrophotometrically at 242 nm. Each experiment was performed in triplicate.

### Experimental design for formulation of nanoemulsion

Bearing in mind the efficiency of the mixture experimental design for the optimization of the NE, the pseudo-ternary phase diagrams was utilized to study and statistically optimize the effects of three components in 8 runs in randomized order. Surfactant and co-surfactant (Smix) in each group were mixed in different volume ratios Span 40: PEG 400 (1:1, 1:2, 1:3, 3:1, 2:1) and the stock of 100 mL of each groups was prepared. These (Smix) ratios were chosen in increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams for the nanoemulsions formation. Construction of pseudo-ternary phase diagrams different ratio of oil and Smix was taken castor oil: Span 40 + PEG 400 (1:2, 2:1, 3:1) and prepared the nanoemulsion. The different conc. of oil and mixture of surfactant and cosurfactant were taken as 0.5:9.5, 0.5:9, 2.8, 3.7, 4:6, 5:5, 6:4 and 7:3. Ternary mixtures were formed in this ratio and quantity of water forming transparent solution was plotted in the pseudo-ternary phase diagram.

### Formulation of acyclovir nanoemulsion according to mixture design

After the development of phase diagram, eight formulations each containing 10 mg ACV, were prepared with varying concentrations of oil mixture, surfactant mixture and cosurfactant mixture (Table 1). For any mixture, the sum of the three components always equals 100%.

**Table 1 Formula composition of prototype formulations**

Oil: Smix	Formulation code	Oil (mg)	Surfactant(mg)	Co-surfactants(mg)
0.5:9.5	F1	0.050	0.470	0.470
0.5:9	F2	0.056	0.467	0.467
2.8	F3	0.198	0.396	0.396
3.7	F4	0.298	0.346	0.346
4:6	F5	0.396	0.297	0.297
5:5	F6	0.496	0.247	0.247
6:4	F7	0.594	0.198	0.198
7:3	F8	0.694	0.148	0.148

### Evaluation of nanoemulsion formulations

#### pH Determination

The pH of each formulation was found before and after dilution by using pH meter results given in Table 3.

#### Thermodynamic stability studies

The obtained NE formulations were subjected to various thermodynamic stability studies in order to assess their phase separation and/or stability<sup>10</sup>. The prepared NE

formulations was subjected to centrifugation at 3,000 rpm for 30 minutes and to repeated cycles of heating and cooling (three freeze-thaw cycles) at -20°C and +25°C to measure their thermodynamic stability. The resulting formulations were then tested for phase separation, creaming, or cracking. The globule size was measured to ensure NE stability.

## Centrifugation

This parameter characterized to check the physical stability of formulation. The nanoemulsion system was centrifuged at 5000 rpm for 10 minutes to determine whether the system shows signs of creaming or phase separation. The system was observed visually for appearance.

## Determination of % drug content in nanoemulsion

The mixture (Nanoemulsion) was centrifuged at 1000 rpm for 15 min, 0.2 ml of supernatant was taken and diluted with 0.1 N HCL. Absorbance was measured at 242nm by UV Spectrophotometer. Concentration of acyclovir was determined using standard curve equation and % drug content was calculated results given in Table 4.

## Zeta potential and vesicle size measurement of optimized batch F3

Zeta Potential of samples was measured by Zeta sizer. Samples were placed in clear disposable zeta cells and results were recorded results show in Fig. 1 & 2

## Formulation development of nanoemulsion loaded gel

**Preparation of carbopol gel base:** 0.5 g Carbopol 934 was weighed and dispersed in water with mild stirring and allowed to swell for 24 hours to obtain 0.5% gel. Later 2 ml of glycerin was added to for gel consistency. Similarly 1 and 2% carbopol gels were prepared.

**Preparation of niosomes gels:** Equivalent to 1g of nanoemulsion formulation was dissolved in 10ml of ethanol and centrifuged at 6000 rpm for 20 minutes to remove the untrapped drug. The supernant was decanted and sediment was incorporated into the gel vehicle.

The incorporation of the nanoemulsion into gels was achieved by slow mechanical mixing at 25 rpm for 10 minutes. The optimized formulation was incorporated into three different gel concentration 0.5, 1 and 2% w/w Table 2.

**Table 2: Composition of different gel base**

Formulation	Carbopol (%)
F1	0.5
F2	1.0
F3	2.0

## Evaluation of nanoemulsion gel formulations

### Determination of pH

Weighed 50 gm of gel formulation were transferred in 10 ml of beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 6-7 to treat the skin infections.

### Spreadability

A modified apparatus suggested was used for determining spreadability. The spreadability was measured on the basis of slip and drag characteristics of the gels. The modified apparatus was fabricated and

consisted of two glass slides, the lower one was fixed to a wooden plate and the upper one was attached by a hook to a balance. The spreadability was determined by using the formula:  $S = ml/t$ , where S, is spread ability, m is weight in the pan tied to upper slide and t is the time taken to travel a specific distance and l is the distance traveled. For the practical purpose the mass, length was kept constant and 't' was determined. The measurement of spreadability of each formulation was in triplicate and the average values are presented.

### Measurement of viscosity

The viscosity of gels was determined by using a Brook Field viscometer DV-II model. A T-Bar spindle in combination with a helipath stand was used to measure the viscosity and have accurate readings. The T-bar spindle (T95) was used for determining the viscosity of the gels. The factors like temperature, pressure and sample size etc. which affect the viscosity were maintained during the process. The helipath T- bar spindle was moved up and down giving viscosities at number of points along the path. The torque reading was always greater than 10%. Five readings taken over a period of 60 sec. were averaged to obtain the viscosity.

### Drug content

Equivalent to 10mg (Acyclovir) of the prepared gel was mixed with 100 ml. of ethyl alcohol. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and the absorbance was measured at 242 nm. Drug content was calculated by linear regression analysis of the calibration curve Table 5.

### In-vitro diffusion study

An *in-vitro* drug release study was performed using modified Franz diffusion cell. Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. Nanoemulsion gel equivalent to 5mg of drug was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at  $37 \pm 0.5^\circ\text{C}$  with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer Table 6-9 & Fig. 3, 4.

## RESULTS AND DISCUSSION

The preliminary study showed that acyclovir is white, crystalline, odorless powder. It is freely soluble in Ethanol, Methanol and 0.1 N HCL soluble in chloroform, slightly soluble in water. The melting point was in the range of  $256-258^\circ\text{C}$  which is in compliance with the standard value of  $256^\circ\text{C}$  as per Indian Pharmacopoeia. From the FT-IR data of the physical mixture it is clear that functionalities of drug have remained unchanged including intensities of the peak. On the basis of above study it was concluded that the solubility in the oils, surfactants and co surfactants like Span 40, Castor Oil, Oleic acid, PEG 400, Ethanol was found to be soluble and Tween 20, Tween 80 and

Sunflower Oil was found to be Slightly soluble for the nanoemulsion preparation of acyclovir. Different physicochemical properties of the selected oils were studied and were found to be favourable for nanoemulsion drug delivery system. The Vesicle size analysis of the optimized formulation F3 was done using particle size analyzer (Horiba). The mean Vesicle size was found to be 41.6nm. Zeta potential of the optimized formulation F3 was determined using particle size analyzer (Horiba). Zeta potential of optimized formulation was found to be -32.4mV. Drug content is most important in nanoemulsion formulation and the data found are satisfactory. It was found to be  $62.25 \pm 0.21$  to  $89.98 \pm 0.25\%$  which shows the good capacity of formulation to hold the drug. Three Different carbopol gel base prepared for optimization (0.5%, 1.0% and 2%) and evaluated for pH, Spreadability, Viscosity measurements and *in vitro* drug release studies. The spreadability was measured on the basis of slip and drag

characteristics of the gels and was in the range of 20.75 – 21.75gms. cm. /sec. *In vitro* drug release study of Optimized formulation was carried out using modified franz Diffusion cell. The optimized formulation F3 showed the maximum 87.980% drug release in 8 hrs.

**Table 3: Results of pH of Acyclovir loaded nanoemulsion**

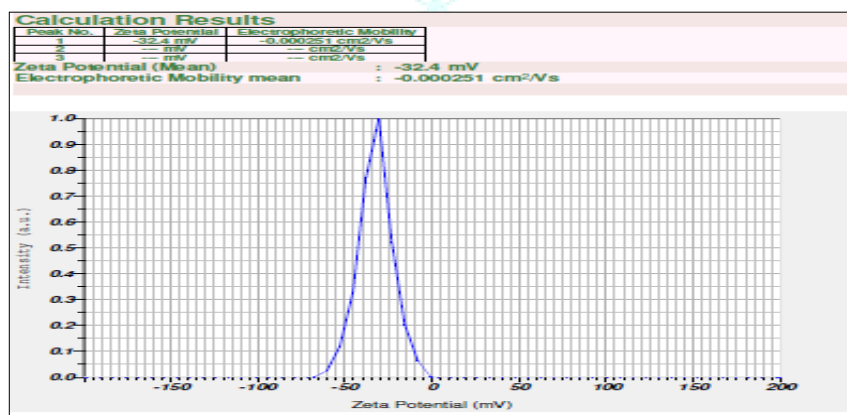
S. No.	Formulation code	pH*
1	F1	6.81±0.02
2	F2	6.92± 0.01
3	F3	7.03±0.02
4	F4	7.04±0.01
5	F5	6.84±0.02
6	F6	6.95±0.03
7	F7	6.98±0.02
8	F8	6.95±0.01

\*Average of three determination

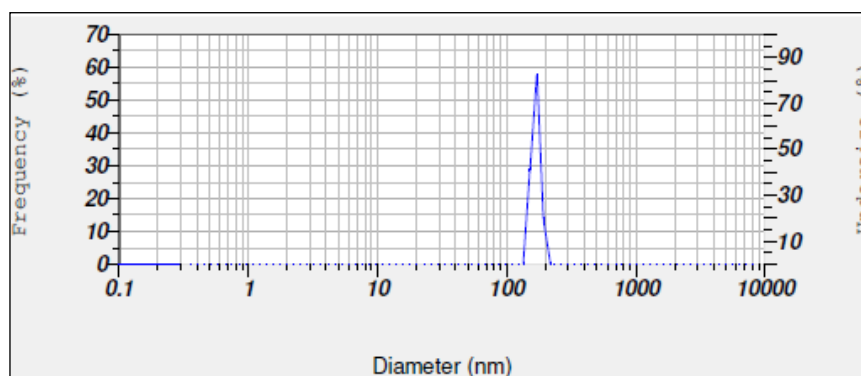
**Table 4: Results of centrifugation and % drug content in nanoemulsion**

Formulation Code	Centrifugation	% Drug Content in nanoemulsion*
F1	Transparent	78.23±0.23
F2	Transparent	75.58±0.15
F3	Transparent	89.98±0.25
F4	Transparent	82.25±0.25
F5	Transparent	70.15±0.65
F6	Transparent	65.56±0.32
F7	Transparent	62.25±0.21
F8	Transparent	65.56±0.28

\*Average of three determination



**Figure 1 Result of zeta potential of optimized batch F3 (-32.4mV)**



**Figure 2 Result of vesicle size of optimized batch F3**

Table 5 Evaluation results of nanoemulsion gel formulations

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
F1	98.89± 0.021	7.0±0.021	20.75±0.075	6231±32
F2	98.68 ± 0.021	7.2±0.040	21.08±0.042	6525±24
F3	99.25 ±0.027	7.0±0.060	21.75±0.059	6758±25

Table 6 *In-vitro* drug release data for formulation F1

Time (h)	Square Root of Time(h) <sup>1/2</sup>	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	13.560	1.132	86.440	1.937
1	1.000	0.000	32.560	1.513	67.440	1.829
2	1.414	0.301	65.560	1.817	34.440	1.537
4	2.000	0.602	75.580	1.878	24.420	1.388
6	2.449	0.778	76.200	1.882	23.800	1.377
8	2.828	0.903	76.210	1.882	23.790	1.376

\*Average of three readings

Table 7 *In-vitro* drug release data for formulation F2

Time (h)	Square Root of Time(h) <sup>1/2</sup>	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.250	1.306	79.750	1.902
1	1.000	0.000	45.580	1.659	54.420	1.736
2	1.414	0.301	68.890	1.838	31.110	1.493
4	2.000	0.602	73.250	1.865	26.750	1.427
6	2.449	0.778	73.560	1.867	26.440	1.422
8	2.828	0.903	74.150	1.870	25.850	1.412

\*Average of three readings

Table 8 *In-vitro* drug release data for formulation F3

Time (h)	Square Root of Time(h) <sup>1/2</sup>	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	18.890	1.276	81.110	1.909
1	1.000	0.000	38.890	1.590	61.110	1.786
2	1.414	0.301	42.560	1.629	57.440	1.759
4	2.000	0.602	54.650	1.738	45.350	1.657
6	2.449	0.778	69.980	1.845	30.020	1.477
8	2.828	0.903	87.980	1.944	12.020	1.080

\*Average of three readings

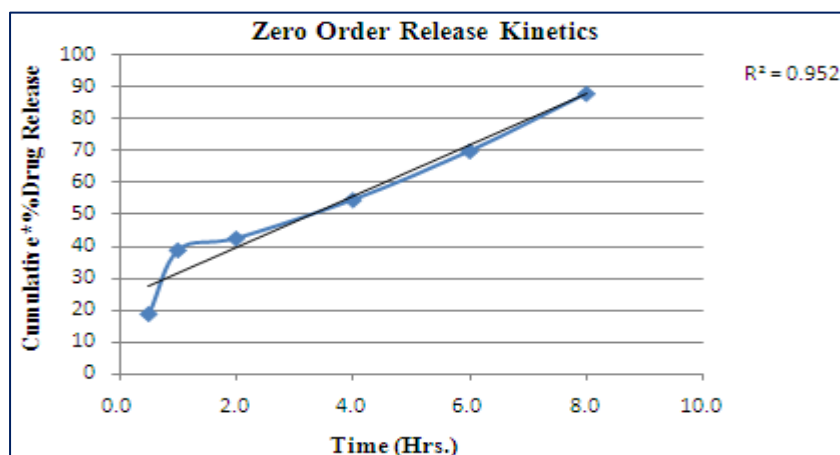


Figure 3 Cumulative % drug released Vs time optimized formulation F3

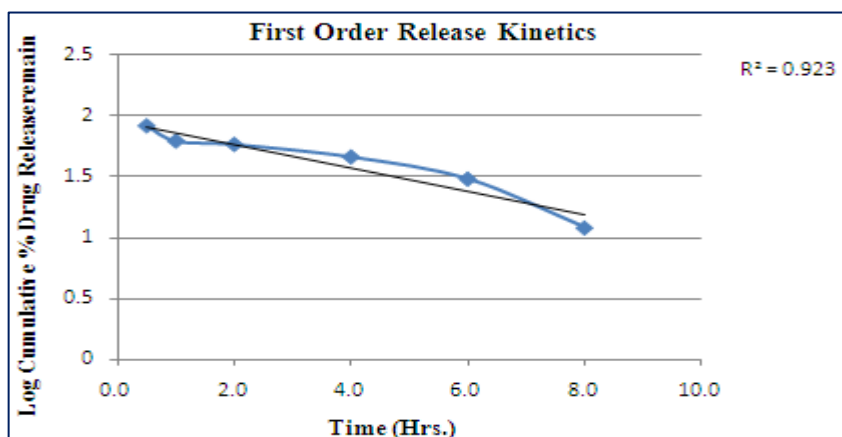


Figure 4 Log cumulative % drug remaining Vs Time optimized formulation F3

Table 9 Regression analysis data of optimized formulation

Batch	Zero Order	First Order
	R <sup>2</sup>	R <sup>2</sup>
F3	0.952	0.923

## CONCLUSIONS

This study demonstrates that a nanoemulsion formulation can be used to improve solubility, bioavailability of Acyclovir and overcome the difficulties associated with its use in the clinic. On analysing saturation solubility study result and pseudo ternary phase diagram, the oil, surfactant and co surfactant were selected. All the prepared formulations exhibited the nanoemulsion properties. The optimised formulation was evaluated for zeta potential, globule size analysis and stability study. The results suggest that the optimised formulation was stable and produced nanoemulsion. The in-vitro diffusion study of the formulation was higher as compared to pure drug, indicating that the prepared formulation is having higher solubility and permeability. Thus it can be concluded that nanoemulsion formulation can be used as a one of

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the formulation technique to enhance the bioavailability of the poorly soluble and permeable drugs. The present work aimed at developing a successful new topical formulation for the delivery of acyclovir was able to increase the efficacy of the currently available commercial products for the topical treatment of Herpes simplex virus infections. In this study, we got success in the development and evaluation of nanoemulsion. Based on higher drug release and lower surfactants concentration, higher solubility as well as higher bioavailability without variable absorption has been optimized as nanoemulsion formulation of acyclovir as oil, surfactants and co-surfactants. The above study leaves a future scope for refining technology which can further be used for preparation of various other nano systems in pharmaceutical products.

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## DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.