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Formulation Development and Evaluation of Ethosomal Gel of Acyclovir for the Treatment of Herpes Zoster

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ABSTRACT

The present study is to develop and evaluate an ethosomal gel formulation of acyclovir (ACV). It aims to provide a topical treatment for many viral infections that affect the skin. Administration of medications topically having the facility of delivering a high concentration of the drug to the skin than would be possible with systemic therapy. Topical administration of drugs is better for local action and the efficiency of the topically administered drug is increased with liposome, proliposomes and ethosomes. Recently, it was found that ethosomal carriers were phospholipid vesicular systems having relatively high concentrations of alcohol, enhances dermal and transdermal delivery of both lipophilic as well as hydrophilic molecules. ACV is the common antiviral agent that is used to treat infections caused by certain types of viruses. It treats cold sores around the mouth (caused by herpes simplex), shingles (caused by herpes zoster) and chickenpox. ACV 5% cream is used for the treatment of infection along with oral dosage forms like tablets. These formulations show poor therapeutic outcome due to the poor bioavailability (15-20%) of oral dosage forms. Ethosomes were formulated using phospholipid, ethanol, polyethylene glycol and purified water by cold method. Ethosomes were evaluated for vesicle size, shape, optical microscopy, entrapment efficiency and *in-vitro* release study. AEF5 have better drug entrapment efficiency than the other formulation. The best formulation (AEF5) was used to prepare gel by using carbopol 934 as a gelling agent. The ethosomes were entrapped in gel matrix of carbopol 980 in different concentration 0.5%, 1.00% and 1.5% w/w. FT-IR studies revealed no interaction between the drug and excipients. The formulated gel formulation was evaluated with parameter pH, viscosity, spreadability, *in-vitro* release test, washability, extrudability study and stability studies. The formulation AEG2 have better *in-vitro* drug release profile which contains carbopol 980 concentration 1%w/w. The present work also focuses on making the formulation more pharmaceutically acceptable.

Keywords: Acyclovir, Ethosomal gel, Viral infections, Phospholipid, % Entrapment efficiency, Vesicle size

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INTRODUCTION

In the past decades, topical delivery of drug by liposomal formulation have evoked considerable interest, it has been evident that traditional; liposomes are of little or no value as carrier for transdermal delivery of drug, because they do not deeply penetrate skin but remains confined to upper layer of the stratum corneum. To overcome problem of poor skin permeability *Cave et al* and *Touitou et al* recently introduce two new vesicular system transferosomes and ethosomes incorporated edge activator (surfactant) and penetration enhancer (alcohols and polyols) respectively to influence the properties of vesicles and stratum corneum[1-3]. Ethosomes are soft malleable vesicles composed mainly of phospholipid, ethanol (relatively high concentration) and water. These soft vesicles represents novel vesicular carrier for enhanced delivery to/through skin. The size of ethosome vesicles can be modulated from tens of microns to nanometres. This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability[4]. The high concentration of ethanol makes the ethosome unique, as ethanol is known for, its disturbance of lipid bilayer organization; therefore when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also because of their high ethanol concentration, lipid membrane is packed less tightly than conventional vesicles, but has equivalent stability allowing a more malleable structure and improves drug distribution ability in stratum corneum lipid. As compared to classical liposomes that delivered drug to outer layers of skin, ethosomes were shown to enhance permeation through the stratum corneum barrier[5]. *Horwtiz et al* in a two armed, double blind, randomized clinical study, compared the efficacy of 5% ACV in a novel liposomal carrier (ethosomes) was compared to that of a commercial 5% ACV cream (Zovirax Cream) and reported that a 5% ACV ethosomal preparation showed significant improvement in treatment of herpetic infection [6]. *Dayan et al* investigated the

delivery of trihexyphenidyl HCl (THP) from ethosomes versus classic liposomes and concluded that in comparison to standard liposomes, ethosomes had higher entrapment efficacy and a greater ability to deliver entrapped fluorescent probe to the deeper layer of skin. The flux of THP through nude mouse skin from THP ethosomes was higher than liposomes[7]. Similarly *Lodzki et al.* designed a transdermal delivery system for cannabidiol by using ethosomal carrier, concluded that transdermal application of ethosomal cannabidiol prevented the inflammation and edema induced by sub plantar injection of carrageenan in the same animal model. Thus ethosomes enabled cannabidiol skin permeation and its accumulation in a depot at level that demonstrated[8]. *Touitou et al* prepared ethosomal drug delivery system of testosterone and minoxidil and studied the depth of skin permeation and demonstrated that the ethosomes enhanced the delivery of drug to skin in terms of both depth and quantity of skin permeation of testosterone and minoxidil[9]. Therefore, reliable drug delivery systems providing better drug penetration can result in better efficacy and also help in the prevention of development of resistance. The aim of the present study was to statistically optimize the ethosomal gel for enhanced skin delivery of ACV which was effective candidate for the treatment of viral infection.

MATERIALS AND METHODS

Material

Acyclovir was obtained as a gift sample from Macleods Pharmaceuticals, Mumbai. Phospholipid was purchased from Himedia Laboratory, Mumbai. Ethanol, propylene glycol and carbopol-934 purchased from CDH chemical Pvt. Ltd. New Delhi. Dialysis membrane of Mol Wt cutoff 1200 was purchased from Himedia Laboratory, Mumbai. Double distilled water was prepared freshly and used whenever required. All other ingredients and chemicals used were of analytical grade.

Determination of λ_{max} of ACV

Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.4 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 7.4 pH buffer solution prepare suitable dilution to make it to a concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Labindia-3000+). A graph of concentration Vs absorbance was plotted.

Preparation of ACV ethosomes

Ethosomal formulations were prepared by using the cold method. This is the most common and widely used method for the ethosomal preparation. Phospholipid and drug and other pharmaceutical ingredient listed in table were dissolved in ethanol in a covered vessel at room temperature with vigorous stirring. This mixture was heated to 30^0 C ± 1^0C and a fine stream of distilled water was added slowly, with constant mixing at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 30^0C ± 1^0C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 4^0 C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator[9]. Six ethosomal formulae were presented in Table 1.

Table 1 Different composition of ethosomes formulation

F. Code	Drug (mg)	Phospholipid (mg)	Ethanol (ml)	PEG (mg)	Water (ml)
AEF1	500	250	5	20	10
AEF2	500	500	5	20	10
AEF3	500	750	5	20	10
AEF4	500	250	10	20	10
AEF5	500	500	10	20	10
AEF6	500	750	10	20	10

Evaluation of ACV loaded ethosomes

Microscopic observation of prepared ethosomes

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared ethosomes formulation.

Surface charge and vesicle size

The vesicles size and size distribution and surface charge were determined by Dynamic Light Scattering method (DLS) (Malvern Zetamaster, ZEM 5002, Malvern, UK).

Zeta potential

The zeta potential was calculated according to Helmholtz–Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm.

Entrapment efficiency

Entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium. About 1 ml of the drug loaded ethosomes dispersion was placed in the Ependorf tubes and centrifuged at 17000 rpm for 30 min. The ethosomes along with encapsulated drug were separated at the bottom of the tubes. Plain ethosomes without drug was used as blank sample and centrifuged in the same manner. In order to measure the free drug concentration, the UV absorbance of the supernatant was determined at 242 nm.

Preparation of ethosomal gels

The incorporation of the acyclovir loaded ethosomes (equivalent to 5%) into separate 10gm gels was achieved by slow mechanical mixing at 25 rpm for 10 minutes. The optimized formulation was incorporated into three different carbapol gel concentration 0.5, 1 and 1.5% w/w. The composition of acyclovir gel formulation was given in table 2.

2 Different composition of ACV gel formulation

S. No.	F. Code	Carbopol gel
1.	AEG1	0.5%
2.	AEG2	1%
3.	AEG3	1.5%

Evaluation of gel

Physical characteristic

The Physical Characteristic was checked for gel formulations (homogeneity and texture).

Determination of pH

The pH of the gels was determined by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

Extrudability study

The gel formulations were filled into collapsible metal tubes or aluminium collapsible tubes. The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Spreadability

An important criterion for gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which the gel readily spreads on application to skin. The therapeutic efficacy of a formulation also depends on its spreading value. A special apparatus has been designed to study the spreadability of the formulations. Spreadability is expressed in terms of time in seconds taken by two slides to slip off from formulation, placed between, under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability. It is determine by formula given below.

$$s = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec), m = weight tied to the upper slide (20 grams),

l= length of glass slide (6cms), t = time taken is seconds.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer. The viscosity was measured using spindle no. 6 at 10 rpm and 25^{0}C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 ±/1^{0}C) before the measurements.

***In-vitro* drug release studies using the prehydrated cellophane membrane**

The cellophane membrane approximately 25 cm x 2cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies. The drug release studies were carried out using modified franz diffusion cell. The cellophane membrane was mounted on the Franz diffusion cell. Formulation was applied through donor compartment on the dialysis membrane. Reservoir compartment was filled with 25 ml phosphate buffer of pH 7.4 The study was carried out at 37 ± 1°C and at a speed of 100 rpm for 8 h. Samples were withdrawn from reservoir compartment at 1 h interval and absorbance was measured spectrophotometrically at 242.0nm. Each time the reservoir compartment was replenished with the same quantity of 7.4 pH phosphate buffer[10,11] .

Release kinetics

In order to elucidate mode and mechanism of drug release, the *invitro* data was transformed and interpreted at graphical interface constructed using various kinetic models. The zero order release Eq. (1) describes the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of transdermal systems, matrix tablets with low soluble drugs, coated forms, osmotic systems etc., where the drug release is independent of concentration.

Qt = Qo + Kot (1)

Where, Qt is the amount of drug released in time t, Qo is the initial amount of the drug in the solution and Ko is the zero order release constant

The first order Eq. (2) describes the release from the system where release is concentration dependent e.g. pharmaceutical dosage forms containing water soluble drugs in porous matrices.

log Qt = log Qo + K1 t /2.303 (2)

Where Qt is the amount of drug released in time t, Q is the initial amount of drug in the solution and K1 is the first order release constant.

Higuchi described the release of drug from insoluble matrix as a square root of time as given in Eq. (3)

Qt = KH √t (3)

Where, Qt is the amount of drug released in time t, KH is Higuchi's dissolution constant [12].

The following plots were made: cumulative % drug release vs. time (zero order kinetic models); log cumulative of % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (Higuchi model).

Stability studies

Stability studies were carried out with optimized formulation which was stored for a period of 45 days at 4±1°C, RT and 40±1°C. The particle size of formulation was determined by optical microscopy using a calibrated ocular micrometer.

RESULTS AND DISCUSSIONS

Acyclovir was found to be white off crystalline powder in appearance, odourless and tasteless. The melting point of ACV (pure drug) was found to be 256-258^{o}C; it matches with the standard (256.5 °C). ACV was freely soluble in ethanol, methanol, 0.1 N HCl, slightly soluble in 0.1 N NaOH, distilled water and soluble in chloroform, phosphate buffer pH 7.4. The absorption maxima of ACV were determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer (Labindia UV 3000+) using concentration range of 5-25µg/ml ACV in 7.4 phosphate buffers Fig 1. Vesicle size and zeta potential of the

ethosomes were measured by photon correlation spectroscopy using a Malvern Zetasizer and entrapment efficiency was determined by measuring the concentration of unentrapped free drug in aqueous medium by UV spectrophotometer the results shown in Table 3. Zeta potential of optimized ethosomes formulation (AEF5) was found -20.5 mV Table 4. Results of evaluation of ethosomal gel formulation (AEG1- AEG3) of optimized formulation (AEF5) were incorporated into three different carbapol gel concentration 0.5, 1 and 1.5 % w/w respectively. Formulation AEG2was found to be good Table 5. Results of *In-vitro* drug release from optimized formulation (AEG2) are given in table 6 was found 82.23 after 8 hrs. The *in vitro* drug release data of the formulation was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equation and Korsmeyer's - pappas models in order to determine the mechanism of drug release. When the regression coefficient values of were compared, it was observed that 'r' values of formulation was maximum i.e 0.989 hence indicating drug release from formulations was found to follow zero order model of drug release kinetics. Table 7 and Fig 2, 3.

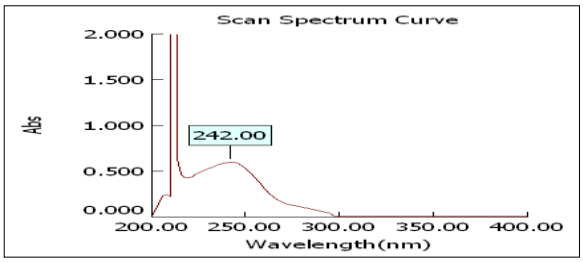

Figure 1 Wavelength maxima of ACV in phosphate buffer pH 7.4

Table 3 Result for vesicle size and entrapment efficiency of drug loaded ethosomes

Formulation Code	Vesicle size (mm)	% Entrapment efficiency
AEF1	458.56±0.23	69.98±0.25
AEF2	426.65±0.45	65.58±0.74
AEF3	433.22±0.52	63.32±0.65
AEF4	415.65±0.36	70.12±0.58
AEF5	331.69±0.65	79.98±0.41
AEF6	485.45±0.58	68.12±0.25

Table 4 Characterization of optimized formulation of ethosomes

Formulation Code	Vesicle size (nm)	Entrapment Efficiency	Zeta potential (mV)
AEF5	331.6	79.98±0.41	-20.5

Table 5 Results of evaluation of gel formulation

Code	Homogeneity and texture	pH	Spreadability (gm.cm/sec.)	Viscosity (cps)	% Assay
AEG1	**Particle present**	6.80±0.05	12.56±0.45	2225±10	98.56±0.45
AEG2	**Smooth**	7.12±0.03	11.23±0.32	2365±15	99.12±0.23
AEG3	**Smooth**	6.95±0.06	10.25±0.25	2456±23	98.45±0.56

Table 6 *In Vitro* drug release data for AEG2

Time (H)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
0.5	0.707	-0.301	24.56	1.390	75.44	1.878
1	1	0	30.25	1.481	69.75	1.844
2	1.414	0.301	42.23	1.626	57.77	1.762
4	2	0.602	55.65	1.745	44.35	1.647
6	2.449	0.778	68.89	1.838	31.11	1.493
8	2.828	0.903	82.23	1.915	17.77	1.250

* Average of three determinations

Table 7 Regression analysis data of ethosomal gel formulation

Formulation	Zero order	First order
AEG2	$R^2 = 0.989$	$R^2 = 0.982$

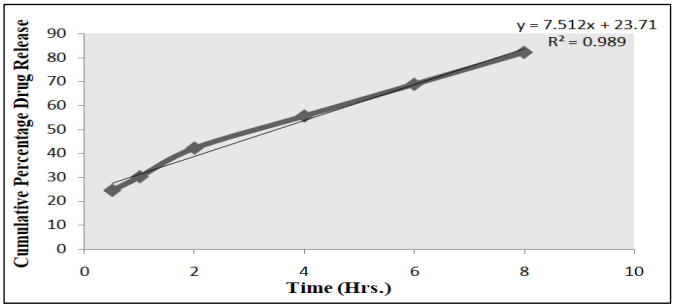

Figure 2 Cumulative percent drug released vs time

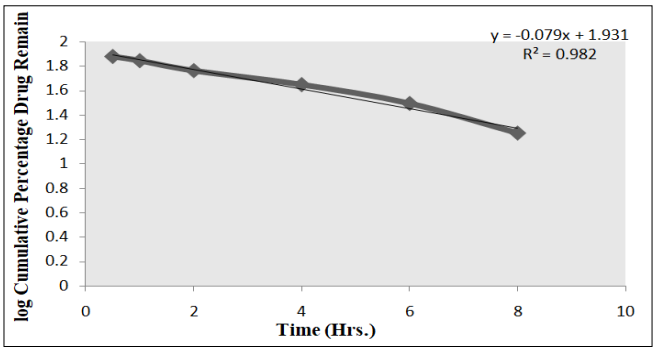

Figure 3 Log Cumulative percent drug remaining vs time

CONCLUSION

Ethosomes of ACV were prepared successfully by using different concentrations of phospholipids and ethanol as well as the incorporation of the ethosomes into carbopol 934 base gel to obtain ethosomal gel formulations. The prepared formulations were characterized for various properties. The compositions of ethosomes and gels were manipulated to investigate their effects on the characteristics of final formulations. It can serve as a useful vehicle for the delivery of ACV through the affected part of the skin for extended period of time. This study also revealed that ethosomal gel (**AEG2**) resides at targeted site for a relatively longer period of time with a zero order release profile. It signifies the improved patient compliance.

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