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Design And Characterization Of Zanamavir Ethosomal Drug Delivery System To Enhance The Bioavailability Via Topical Drug Delivery

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Abstract

The present study is to develop and evaluate an ethosomal gel formulation of Zanamavir . 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. 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. **ZEF7** have better drug entrapment efficiency than the other formulation. The best formulation (**ZEF7**) 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 **ZEF7** have better in-vitro drug release profile which contains carbopol 980 concentration 1.5 %w/w. The present work also focuses on making the formulation more pharmaceutically acceptable.

Keywords: Zanamavir, Ethosomal gel, Phospholipid, % Entrapment efficiency, Vesicle size

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 corneum1-3. 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 deformability4. 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 barrier5. Ethosomes enabled cannabidiol skin permeation and its accumulation in a depot at level that demonstrated8. 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 minoxidil9. 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 ZANAMAVIR which was effective candidate for the treatment of viral infection.

MATERIALS AND METHODS

Zanamavir 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 cut off 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.

EXPERIMENTAL METHODOLOGY

Preparation of standard stock solution of Zanamivir:

Standard stock solution of Zanamivir (1mg/mL) was prepared by transferring 10 mg of Zanamivir into a 10 mL volumetric flask containing 4 mL of (8:2) methanol and water. It was then sonicated for 15 minutes and solution was diluted up to the volume by methanol and water. From these, further dilutions were made using (8:2) methanol and water to produce solution of Zanamivir (100 µg/mL).

Method of preparation of Ethosomes:

Preparation of Sonicated Zanamivir Ethosomes (by Cold method):

The Zanamivir Ethosomal system comprised of 20-40% of ethanol, 10% of propylene glycol, 2-7% of phospholipids, 0.005g of cholesterol and an aqueous part of 100%w/w. At room temperature, 0.020g of Zanamivir was added to ethanol in a covered vessel along with propylene glycol and dissolved by stirring it vigorously. At 30^{o}C mixture was heated using separate vessel and then drop wise it was added to the mixture in the centre of the vessel by stirring it at 700 rpm for 5min in a vessel which was covered.

Then by using extrusion[5] method or sonication[5] method the particle size of Ethosomal formulation was reduced to the desirable extent. At last the Ethosomal formulation was kept under refrigeration. The following process was used to prepare Ethosomes spontaneously.

Preparation of Unsonicated Zanamivir Ethosomes:

The Ethosomes consisted of 2 – 7% phospholipids, 20-40% ethanol, 0.005 % of cholesterol, 0.020g of Zanamivir and distilled water quantity sufficient 100% w/w at room temperature. The mixture was heated to 30^{o}Cand continuous stirring with 700RPM for 5 minutes is added drop wise in a closed i.e. minutes vessel for 5 min.

Preparation of Ethosomal gel of Zanamivir

In the Carbopol gel of various compositions (1%, 1.5%, 2%, 2.5%, 3%, 3.5% w/w) the best achieved Ethosomal suspension, of formula was selected and was incorporated specific amounts of Carbopol 934 powder which were added slowly to ultra pure water and kept for 20mins at a temperature of 100^{o}C. Then later on, drop wise Triethanolamine was added into it. Then incorporation of an accurate quantity of formula which contains Zanamivir (1.5% w/w) was done in the gel base and then water of sufficient quantity was added with continuous stirring to the other ingredients of the formulation until a formulation of homogenous nature was obtained which were coded as (G-1, G-2, G-3, G-4, G5, G6).By the use of 1.5%w/w Carbopol, a gel constituting free Zanamivir was prepared with the aid of similar method.

Table 1. formulation of Zanamavir Ethosomes

S.No	Ingredients	Sonicated Ethosomes					
		ZEF1	**ZEF2**	**ZEF3**	**ZEF4**	**ZEF5**	**ZEF6**
1	Drug (g)	0.020	0.020	0.020	0.020	0.020	0.020
2	Lecithin (Soya lecithin %)	2	3	4	5	6	7
3	Ethanol (%)	20	30	40	20	30	40
4	Propylene Glycol (%)	10	10	10	10	10	10
5	Cholesterol (g)	0.005	0.005	0.005	0.005	0.005	0.005
6	Water	QS	QS	QS	QS	QS	QS
S.No	**Ingredients**	**Unsonicated Ethosomes**					
		ZEF7	**ZEF8**	**ZEF9**	**ZEF10**	**ZEF11**	**ZEF12**
1	Drug (g)	0.020	0.020	0.020	0.020	0.020	0.020

2	Lecithin (Soya lecithin %)	2	3	4	5	6	7
3	Ethanol (%)	20	30	40	20	30	40
4	Propylene Glycol (%)	10	10	10	10	10	10
5	Cholesterol (g)	0.005	0.005	0.005	0.005	0.005	0.005
6	Water	QS	QS	QS	QS	QS	QS

Table 2. Formulation of Zanamavir Ethosomal Gel

Gel Formulation	Zanamivir Ethosomal suspension (ml)	Carbopol (%)	Triethanolamine(ml)	Phosphate Buffer (pH 7.4)
G-1	0.020 g	1	0.5	Q.S
G-2	0.020 g	1.5	0.5	Q.S
G-3	0.020 g	2	0.5	Q.S
G-4	0.020 g	2.5	0.5	Q.S
G-5	0.020 g	3	0.5	Q.S
G-6	0.020 g	3.5	0.5	Q.S

CHARACTERIZATION OF ETHOSOMES

SIZE AND SHAPE ANALYSIS:

For the determination of Ethosomes, average size microscopic analysis was done. A sample of Ethosomes was taken and it was diluted by using distilled water. For the examination at 45×15 X microscope on a glass slide diluted drop is taken which is then covered with a cover slip. By using calibrated eyepiece micro meter with stage micro meter the diameter of 150 vesicles was determined. The formula used for calculating average diameter was

Average diameter = nd /n
Where
n= number of vesicles
d= diameter of vesicles

SCANNING ELECTRON MICROSCOPY:

Determination of morphological characters like its texture of smoothness, round structure and aggregate gel formation of Zanamivir Ethosomes was done using scanning electron microscopy [8, 9, 10]

ENTRAPMENT EFFICIENCY:

A 10ml aliquot of Zanamivir Ethosomal suspension was ultra centrifuged to determine entrapment efficiency. For every 2minutes gap all the samples were vortexes for 5minutes each cycle at least of 2 cycles were done. Then from all the samples which have been vortexed a quantity of 1.5ml is taken and Ethosomal formulations which are fresh and untreated were put into different tubes of centrifuge. The centrifugation of the samples was done at a rate of 20,000 rpm for duration of 3 hours. Separation of the supernatant layer was done and diluted with a suitable quantity of water and then absorbencies' for the respective concentration of the drug were found at 242nm in both un-vortexed and vortexed samples[11]. The efficiency of entrapment was calculated accordingly:

Entrapment efficiency=T-C/T ×100

Where

T denotes detected total drug from the vortexed supernatant sample.

C denotes unvortexed detected drug supernatant sample and amount of drug entrapped.

CHARACTERIZATION OF GEL:

Surface morphology:

The scanning electron microscope was employed by utilizing the gold sputter technique to detect the morphology of the surface of Ethosomes was done. The system was dried under vacuum and coated with gold palladium and then microscopically observed.

Organoleptic Characteristics:

The psycho rheological properties of the formulations were tested like the colour, odour, phase separation, feel upon application (greasiness, grittiness) and texture.

Wash ability:
On to the skin a small amount of gel was applied and then it was washed with water and checked whether the gel was able to be totally washed or not.

Spread ability:
The determination of Spread ability was done by glass slide apparatus and modified wooden block. A specified amount of the gel was put over the movable pan with a slide of glass affixed to it. It was then kept on the fixed slide made of glass so that the gel was sandwiched in between the two slides of glass for duration of 5min. The continuous removal of weight was also done. The determination of Spread ability was done by using the following formula:
S=M/T
where,
T denotes Time in seconds,

M denotes Mass in grams,
S denotes Spread ability in g/s,

pH:
A solution of 1gm of gel was prepared which was dissolved in distilled water of 30ml with a pH 7. By bringing contact of the probe of the pH meter with the samples in a digital pH meter the pH of the Ethosomal gel was determined.

Content uniformity and Drug content:
Dissolve 1gm gel in 100ml of pH of 7.4 phosphate buffer for duration of about 48hrs and by using magnetic stirrer constantly stir the mixture. Then filtration of the solution was done and UV spectrophotometer was used to observe it at a maximum wavelength of 242nm. Triplicate measurements were made [12, 13, 14]

DRUG RELEASE STUDY FROM RAT SKIN:
Specifically improvised laboratory model of Franz diffusion cell. According to the literature studies of Zanamivir Ethosomes, formulation of Skin permeation is carried out. The receptor cell volume was 20ml and the area of permeation of the cell which was effective was about 2.4cm2 and 37 ±0.50c temperature is adjusted. Using the Magnetic stirrer at 100RPM the 20ml of solution whose pH is 7.4 is continuously stirred. Rat Skin is placed in between donor and receiver. On the rat skin was placed, 1gm of formulation of Ethosomes is applied on diffusion cell maintained with constant stirring and it is also applied on epidermis skin of the rat. Then samples of 1ml quantity were taken out of diffusion cell receptor compared at predetermined time intervals. They were analysed by spectrophotometer method at 305 nm after a suitable dilution. Immediate replenishment of the receptor phase with a volume of equal quantity of fresh pH 7.4 buffer was done. For the permeation studies of the skin, triplicate experiments were conducted.

EVALUATION TESTS

1. Scanning electron microscopic studies for vesicle- Filter membrane-interaction study:

A filter membrane with a pore size of 50nm was applied with a vesicle suspension of (0.2ml) and then introduced into the diffusion cell. The upper side was the one which air exposed. Whereas the lower side container pH 6.5 (Saline buffer solution of Phosphate). Filters were removed after an hour and preparation was done for SEM studies by keeping it overnight in Karnovsky's fixative for fixation at 4 degree C and then by using ethanol graded sol.(100% 95%,90%,70%,50%,30%v/v in water) it was dehydrated. Finally filters were put a coating of gold and its examination was done in SEM (Leica, Germany, Bensheim).

2. Skin permeation studies:

By the use of a pair of scissors Wistar rats hairs were cut short carefully (<2mm) and then by the use of a scalpel the abdominal skin and the underlying connective tissue was separated from each other. On aluminium foil the excised skin is kept and the removal of subcutaneous and adipose tissue was done, which acts like diffusion cell with an area of diffusion 1.0cm 2 and it has 10 ml effective permeation volume at 32±10 C. Temperature and PBS (10ml of pH 6.5) buffer. After placing the skin

between compartment receptor and compartment donor, Ethosomal formulation of 1.0ml was applied on skin epidermis. About Samples of 0.5ml were withdrawn from the diffusion cell at various time intervals of about 1,2,4,8,12,16,20,24 hrs and the samples were assayed by HPLC method.

3. Stability study:

By storing the particles at 4 ^{o}C the determination of their stability was done. After duration of about 180 days the particle size, zeta potential and entrapment efficiency are estimated by the earlier described methods.

In-vitro release kinetics:

The dissolution pattern obtained for all the formulations were graphically plotted for the following parameters namely.

• Peppa's model / equation of Korsmeyer – % drug released Log cumulative v/s log time.

• Higuchi's model – Square root of Time v/s Cumulative % drug released.

• First – order kinetic model – time v/s Drug remain log cumulative %.

• Zero - order kinetic model – Time v/s Cumulative % drug released.

RESULTS AND DISCUSSIONS

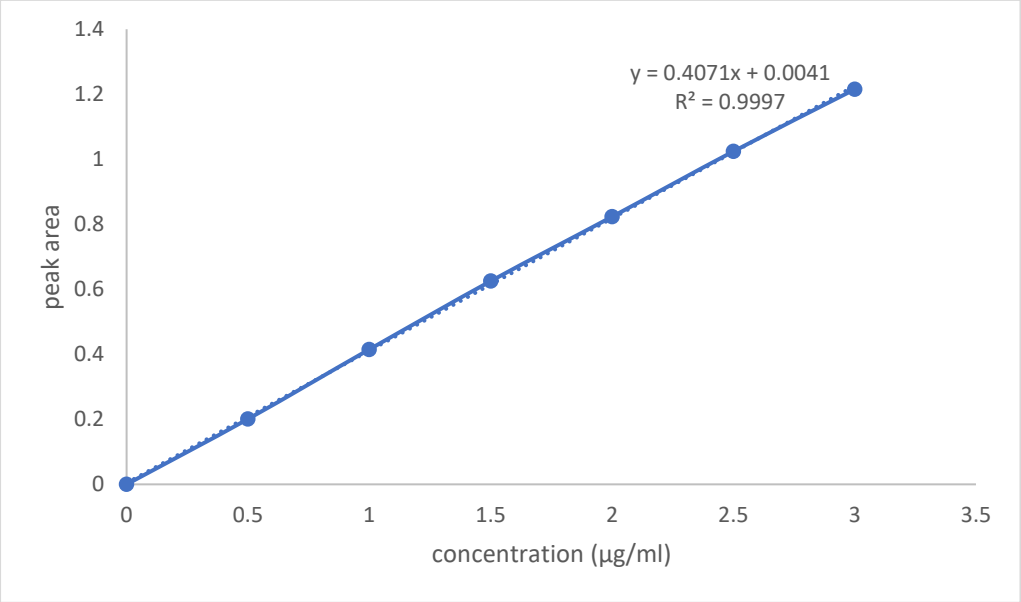
Calibration curve of Zanamivir in methanol (made up with pH 7.4: phosphate buffer)

Standard solutions of different concentration were prepared and their absorbencies were measured at 305 nm. Calibration curve was plotted against drug concentrations versus absorbence as given below.

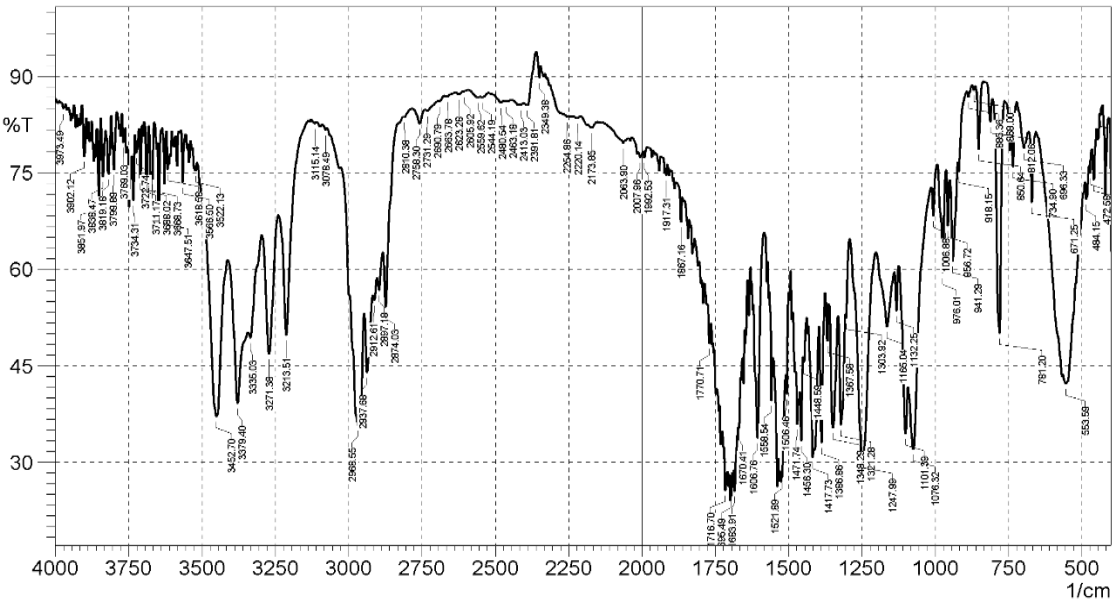
Optimization of Ethosomal formulation:

Ethosomal formulations composed of phospholipids, drug and ethanol were prepared. Ethosomal suspension obtained with Sonication were slight yellowish in colour and hazy in appearance. Different characteristics of Ethosomes and the effect of Sonication were further evaluated and results are reported under the characterization.

Fig 1 **Calibration curve of Zanamivir**



COMPATABILITY STUDIES



pure drug

FIG 2 : FTIR spectrum of pure drug

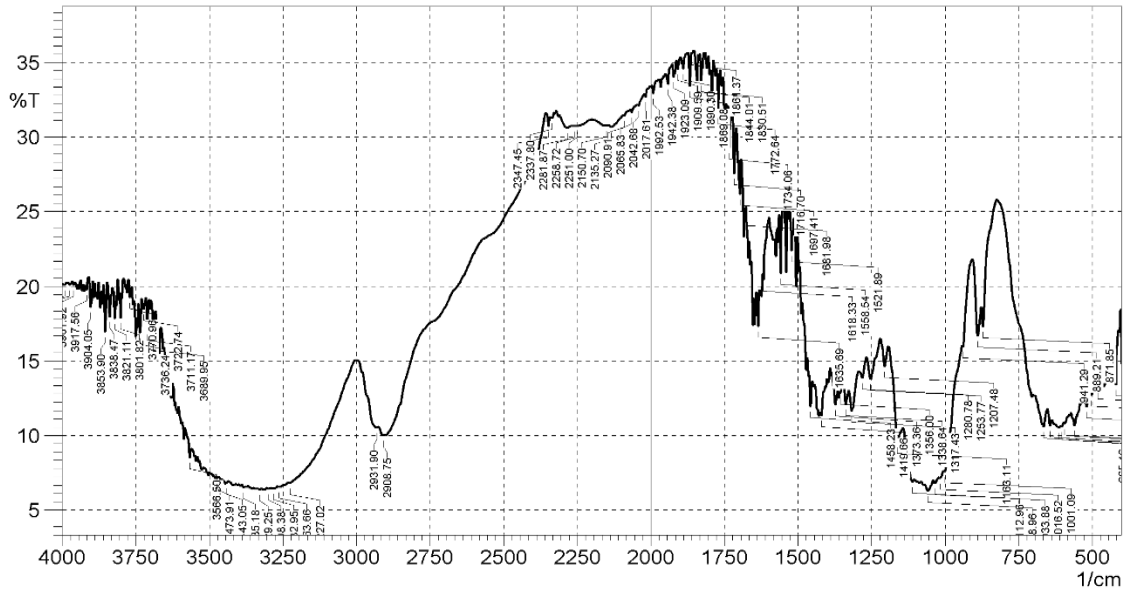


FIG 3 : FTIR spectrum of ethosomal formulation

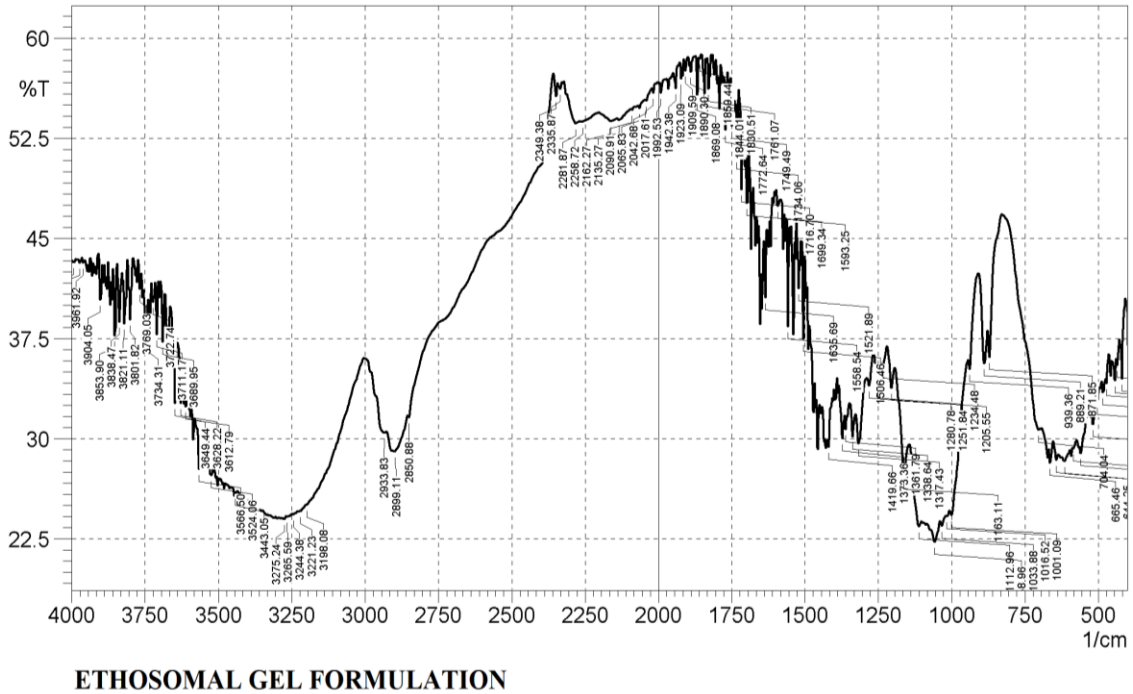


FIG 4 : FTIR spectrum of ethosomal gel formulation

DSC

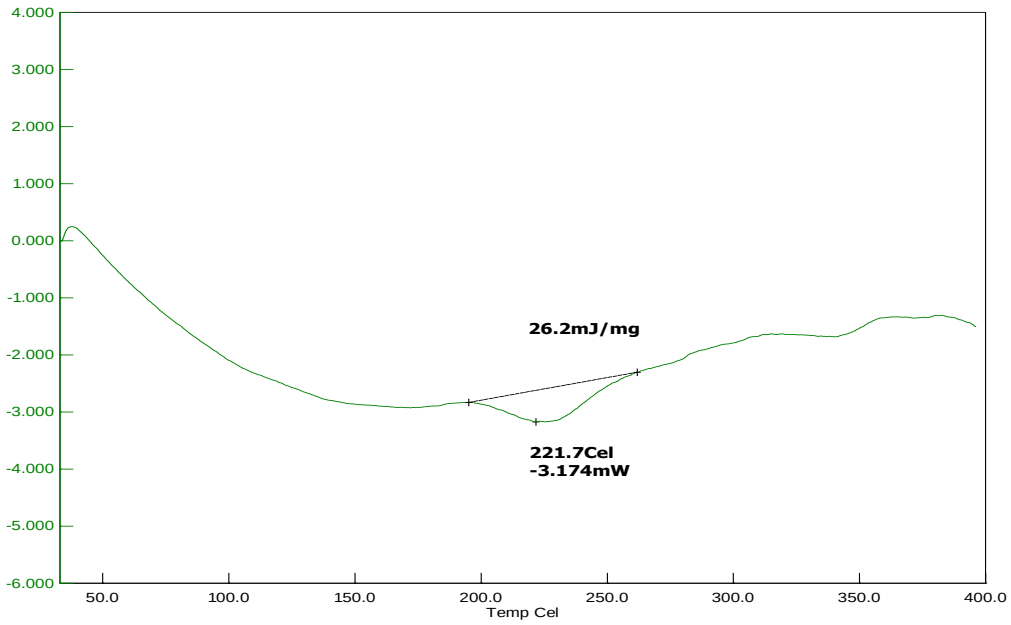


Fig 5: Dsc spectrum of pure drug

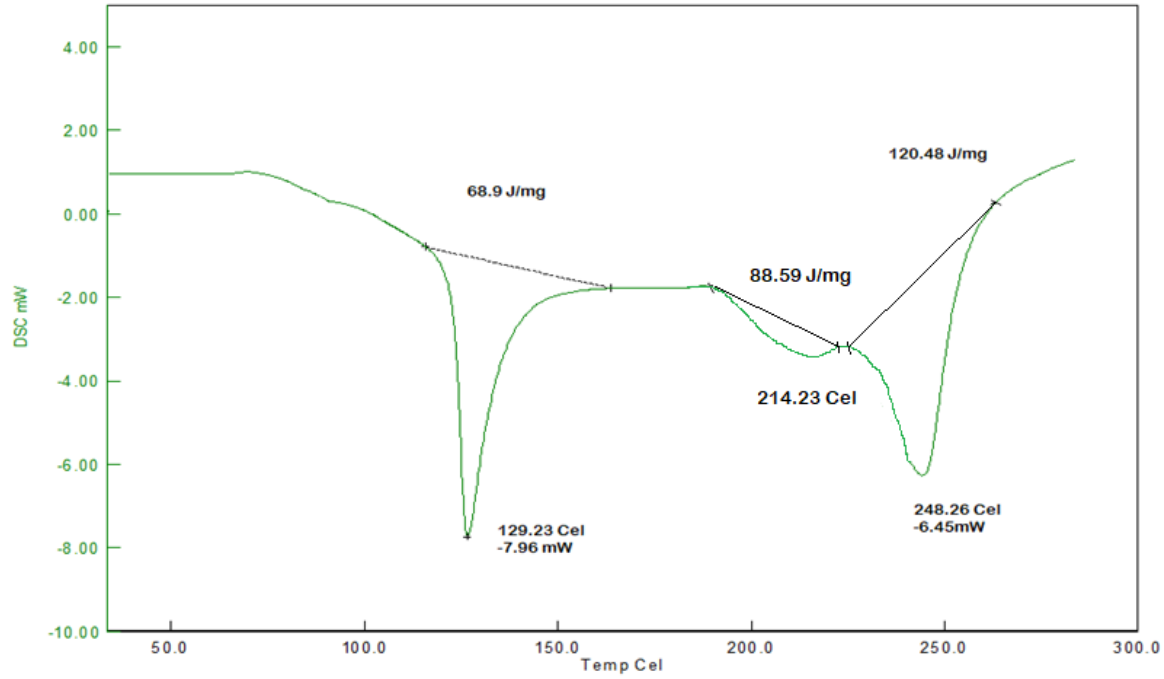


Fig 6 : dsc spectrum of ethosomes

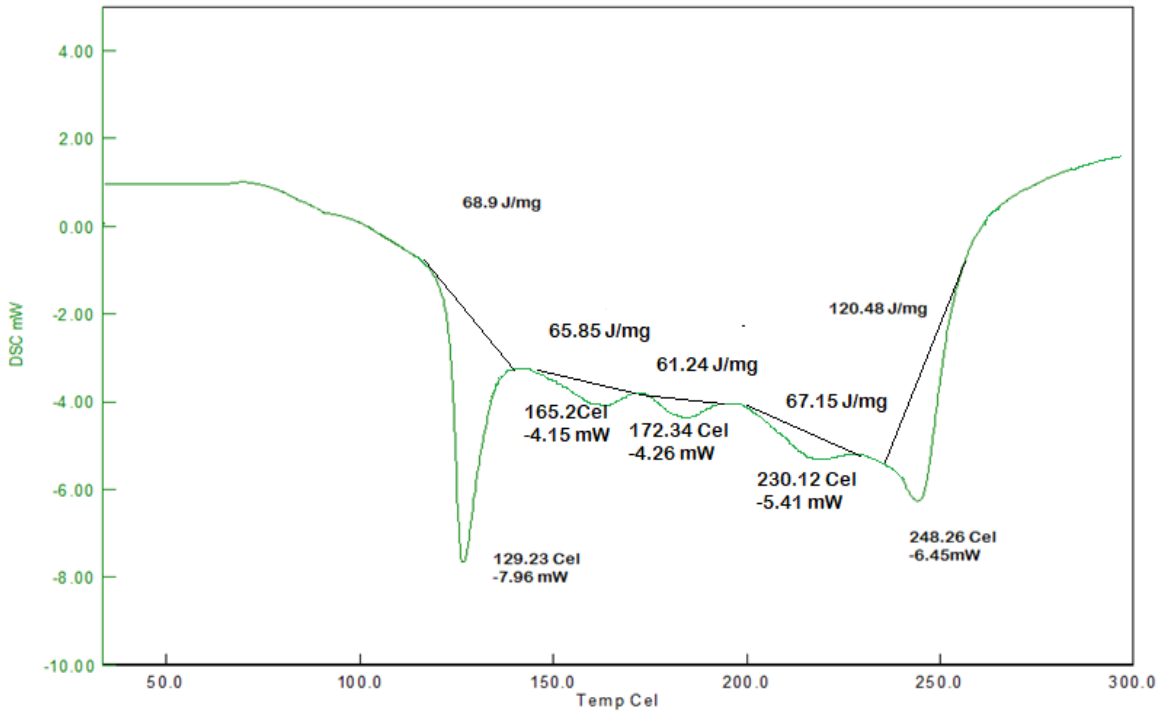


Fig 7 : dsc spectrum of ethosomal gel formulation

CHARACTERIZATION OF ETHOSOMES:

Since the physical characterization is meant for physical integrity of the dosage form, the results were pooled at one place. Discussion on the results described for Sonicated and unsonicated Ethosomes formulations is presented below.

SIZE AND SHAPE ANALYSIS:

Microscopic analysis was performed under different magnification to visualize the vesicular structure, lamellarity and to determine the size of Ethosomal and Liposomal preparations.

ETHOSOMES WITH SONICATION:

Sonication method was adopted to reduce the vesicular size by giving high level energy to the lipid suspension. The type of Sonicator used was probe Sonicator in order to produce high energy to a small aliquot of the lipid suspension. The selected Ethosomal formulations were subjected to Sonication by the probe Sonicator. The photomicrograph revealed reduce the particle size of the Ethosomes after being subjected Sonication. The result of size and shape revealed consistency with the observations of Jain NK et al.,

ETHOSOMES WITHOUT SONICATION:

Ethosomes were prepared comprising of the formula containing 2-5% Phospholipids, 20-40% ethanol and quantity sufficient water were found to appear as multilamellar vesicles. It was observed that the Ethosomal lamellae were evenly spaced to the core. This confirms that the vesicular structure of high ethanolic concentration were present. This Liposomal formulation was observed to be longer than that of Ethosomal formulation which were characterize to be multilamellar and gained type too. Size

distribution and average vesicular size analysis were done as per the procedure mentioned under methodology.

Table 3. Size distribution of Zanamivir formulation ZEF1-ZEF12:

s.no	Average size (d)	No of Vesicles (n*)
ZEF1	55.45	165
ZEF2	62.34	166
ZEF3	63.15	167
ZEF4	71.24	221
ZEF5	58.34	223
ZEF6	48.25	221
ZEF7	68.54	225
ZEF8	66.32	210
ZEF9	61.75	232
ZZEF10	95.36	214
ZZEF11	98.54	154
ZZEF12	102.34	164

ENTRAPMENT EFFICIENCY:

Once the presence of bilayer vesicles was confirmed in the Ethosomal system, the ability of vesicles for entrapment of drug was investigated by ultra centrifugation. Ultracentrifugation was the method used to separate the Ethosomal vesicles containing drug and un-entrapped or free drug, to find out the entrapment efficiency.

Fig 8: Scanning Electron Microscopy: (ZEF7)

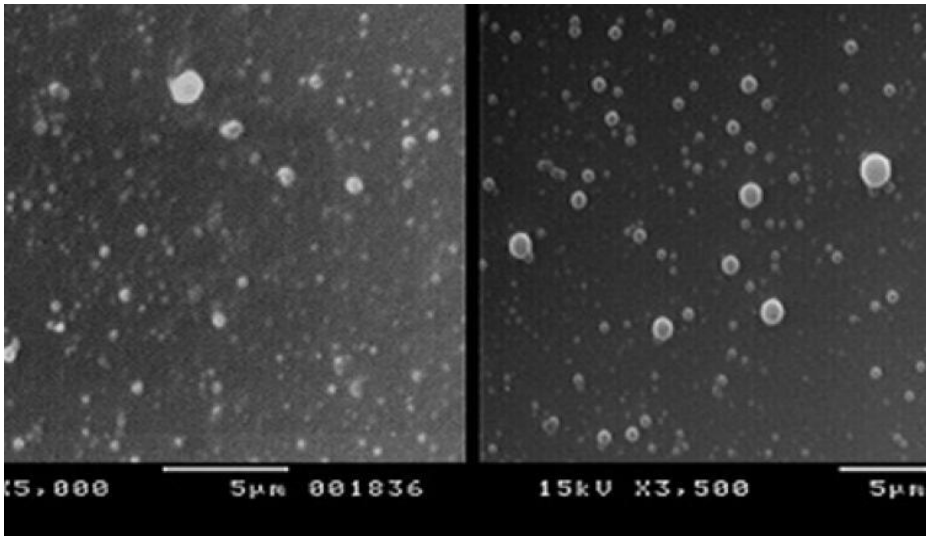


Table 4.Drug entrapment efficiency of various formulations of Ethosomes

S. No	Sample Code	Concentration		Dilution factor	Amount of Drug T=C* DF	Entrapped Drug E=T-U	% Entrapped drug %E=E/T X 100
01	ZEF1	Total Drug (T)	9.86	10	98.6	67.1	68.0
		Free drug (U)	3.15	10	31.5		
02	ZEF2	Total Drug (T)	9.45	10	94.5	72.9	77.1
		Free drug (U)	2.16	10	21.6		
03	ZEF3	Total Drug (T)	9.54	10	95.4	51	53.4
		Free drug (U)	4.44	10	44.4		
04	ZEF4	Total Drug (T)	9.82	10	98.2	64.1	65.2
		Free drug (U)	3.41	10	34.1		

05	ZEF5	Total Drug (T)	9.88	10	96.5	73.1	77.3
		Free drug (U)	2.24	10	23.4		
06	ZEF6	Total Drug (T)	9.27	10	92.7	59.4	64.0
		Free drug (U)	3.33	10	33.3		
07	ZEF7	Total Drug (T)	9.65	10	98.8	76.4	75.7
		Free drug (U)	2.34	10	22.4		
08	ZEF8	Total Drug (T)	9.54	10	95.4	69.8	73.1
		Free drug (U)	2.56	10	25.6		
09	ZEF9	Total Drug (T)	9.57	10	95.7	74.3	77.6
		Free drug (U)	2.14	10	21.4		
10	ZEF10	Total Drug (T)	9.56	10	95.6	59.9	62.6
		Free drug (U)	3.57	10	35.7		
11	ZEF11	Total Drug (T)	9.58	10	95.8	59.3	61.8
		Free drug (U)	3.65	10	36.5		
12	ZEF12	Total Drug (T)	9.88	10	98.8	64.3	65.0
		Free drug (U)	3.45	10	34.5		

The maximum entrapment efficiency of Ethosomal vesicles as determined by ultracentrifugation was 76.4% for Ethosomal formulation containing 20% ethanol (ZEF7) which was almost double to the

formulation containing 40% ethanol (ZEF12). As the ethanol concentration increased from 20% to 40% w/w, there was decrease in the entrapment efficiency and with further increase in the ethanol concentration (>30% w/w) the vesicle membrane becomes more permeable that lead to further decrease in the entrapment efficiency. Results of entrapment efficiency also suggest that 2% phospholipids is optimal concentration for entrapment efficiency and hence increased in concentration of phospholipids reduces the entrapment efficiency of vesicles.

Entrapment efficiency of Ethosomal formulations is significantly different which are reported. Increase in entrapment efficiency may be due to the possible reduction in vesicle size. The detrimental effect on the vesicle during ultra-centrifugation which are larger in size. Sonication gives the more uniform lamellae, smaller vesicle and uniform size and hence it may be the reason for higher vesicular stability and lesser vesicular disruption during ultra centrifugation.

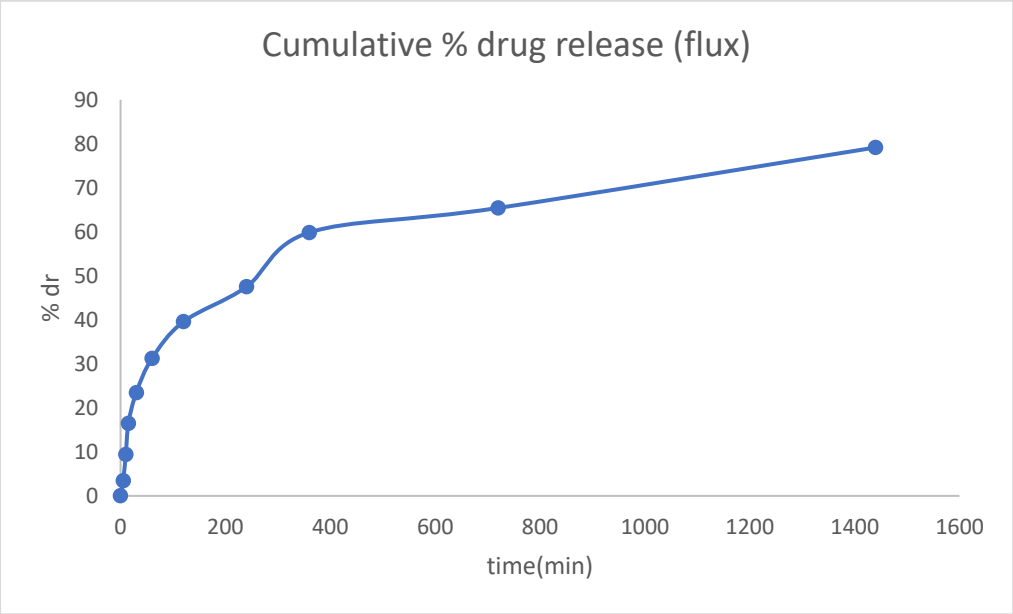
Table 5. Invitro Drug Release Profile for ZEF7 Ethosomes

S.No	Time (min)	Cumulative % drug release (flux)
1	5	3.45
2	10	9.41
3	15	16.48
4	30	23.45
5	60	31.25
6	120	39.57
7	240	47.48
8	360	59.84
9	720	65.41
10	1440	79.15

IN-VITRO SKIN PERMEATION STUDY:

The objectives in the development of in-vitro diffusion tests are to show the release rate and extent of drug from the dosage form. The in-vitro skin permeation study of Carvedilol from Ethosomal formulation was studied using Franz diffusion cell and method described in methodology chapter. The release data was obtained for all the Ethosomal formulations. Spectrometric results were obtained and given consideration to sampling loss, to calculate actual cumulative drug diffused was calculated since the volume of receptor cell was only 20 ml. The obtained diffused amount of drug was extrapolated to diffusion by unit surface area of rat skin. These cumulative values were plotted as a function of time and steady state transdermal flux was calculated from the slop of linear portion.

Fig 6: Invitro Drug Release Profile for ZEF7 Ethosomes



EVALUATION OF ETHOSOMAL GEL

Organoleptic characteristics of Ethosomal gel:

Organoleptic Characteristics	Color: creamy Greasiness: Non-greasy Grittiness: Free from grittiness Skin irritation: No skin irritation.
Washability	Easyily washable without leaving any residue on the surface of the skin.
Spreadability	Easily spreadable.

Drug content and content uniformity (ZEF7)

The drug content for the ZEF7 was found to be 99.13%

Table 6.pH measurement of the Ethosomal Gels

Formulation Code	pH
ZEF1	6.85
ZEF2	6.82
ZEF3	6.83

ZEF4	6.81
ZEF5	6.84
ZEF7	6.85
ZEF8	6.83
ZEF9	6.82
ZZEF10	6.83
ZZEF11	6.81
ZZEF12	6.83

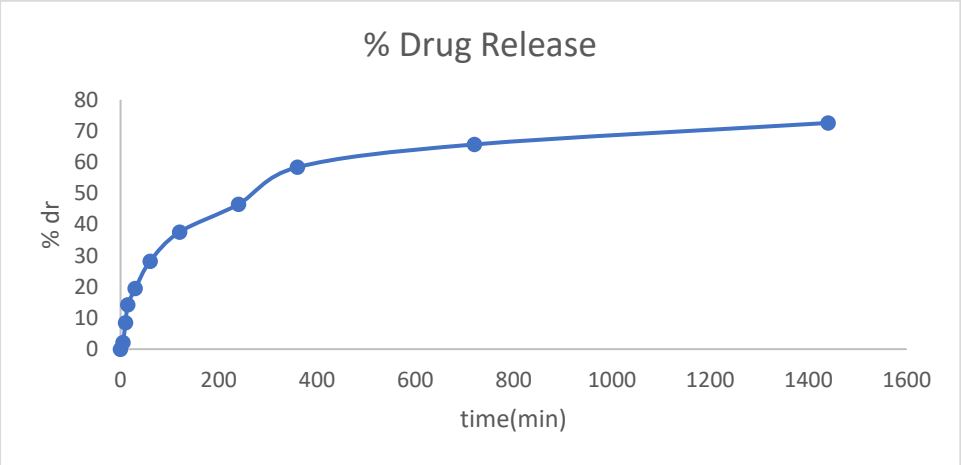


Fig 7.Drug Release Kinetics for ZEF7 Gel:

Table 7.Drug Release Profile for ZEF7 on Rat Skin (Ethosomal Gel)

Time(Min)	Dilution Factor	% Drug Release
0	10	0
5	10	2.14
10	10	8.47
15	10	14.27
30	10	19.42
60	10	28.18
120	10	37.54

240	10	46.46
360	10	58.34
720	10	65.64
1440	10	72.54

STABILITY STUDY:

Ethosomal formulations were observed for any change in appearance or color for a period of 8 weeks. There was no change in appearance in Ethosomal formulations throughout the period of study. Even significance changes were not observed under the magnified view indicating that there was no increase in average size of vesicles for different formulation. The stability of drug was further confirmed by spectral data and there was found to be stable.

Table 8. **Drug Release Kinetics for ZEF7 Gel**

Time (t)	**root (t)**	**Cumulative (%) release q**	**log (t)**	**log(%) release**	**log (%) remain**	**release rate (cumulative % release / t)**	**1/cum % release**	**Peppas log q/100**	**% drug remaining**
0	0	0	0.000	0.000	2.000	0.000	0.0000	0.000	100
5	2.236	2.14	0.699	0.330	1.991	0.428	0.4673	-1.670	97.86
10	3.162	8.47	1.000	0.928	1.962	0.847	0.1181	-1.072	91.53
15	3.873	14.27	1.176	1.154	1.933	0.951	0.0701	-0.846	85.73
30	5.477	19.42	1.477	1.288	1.906	0.647	0.0515	-0.712	80.58
60	7.746	28.18	1.778	1.450	1.856	0.470	0.0355	-0.550	71.82
120	10.954	37.54	2.079	1.574	1.796	0.313	0.0266	-0.426	62.46
240	15.492	46.46	2.380	1.667	1.729	0.194	0.0215	-0.333	53.54
360	18.974	58.34	2.556	1.766	1.620	0.162	0.0171	-0.234	41.66
720	26.833	65.64	2.857	1.817	1.536	0.091	0.0152	-0.183	34.36
1440	37.947	72.54	3.158	1.861	1.439	0.050	0.0138	-0.139	27.46

Table 9.Stability data for optimized formulation

Formulation Code	Drug content in %									
	Initial		After 2 weeks		After 4 weeks		After 6 weeks		After 8 weeks	
ZEF7	4±2 ^{o}C	27±2 ^{o}C	4±2 ^{o}C	27±2 ^{o}C	4±2 ^{o}C	27±2 ^{o}C	4±2 ^{o}C	27±2 ^{o}C	4±2 ^{o}C	27±2 ^{o}C
	100	100	100	99.24	99.16	98.12	98.61	97.86	98.13	97.51

CONCLUSION

Ethosomes of ZANAMAVIR 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 ZANAMAVIR through the affected part of the skin for extended period of time. This study also revealed that ethosomal gel (**ZEF7**) 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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