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Research Article

Formulation And Evaluation of Acyclovir Hydrogel For Topical Delivery

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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.

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 nanoparticles1, 2. ACV is slightly soluble in water, with solubility ranging from 1.2 to 1.6 mg/mL at room temperature3, 4 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 incomplete5.Ethosomes are the tailored form of liposomes that are more in ethanol content. The ethosomal system is composed of phospholipid, ethanol and water. They can permeate the skin and enhance compound delivery both to deep skin stratum and systemically. This ethanol fluidized both ethosomal lipids and bilayers of the stratum corneum intercellular lipidThe soft, flexible vesicles penetrate the disorganized lipid bilayers. Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These „soft vesicles" represents novel vesicular carrier for improved delivery to/through skin. The size of ethosomes vesicles can be modulated from tens to nanometers[4].

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.

Preformulation
Determination of λmax
The absorption maximum of Acyclovir was determined by running the spectrum of drug solution in double beam ultraviolet spectrophotometer.

Procedure
Preparation of stock solution
10 mg of Acyclovir was accurately weighed and transferred to a 10 ml volumetric flask and

volume was made up to 10 ml with 7.4 pH Phosphate buffer saline and shaken to dissolve. This will produce the stock solution having concentration of 1000 μg/ml.

Preparation of secondary stock solution

Now, from the primary stock solution 1 ml of solution was taken and diluted with the 10 ml of 7.4 pH phosphate buffer in a 10 ml of volumetric flask. This will give the secondary stock solution which is having concentration of 100 μg/ml.

Preparation of the aliquots

The different aliquots were prepared having concentration of 5, 10, 15, 20, 25μg/ml with the help of the secondary stock solution with 7.4 pH Phosphate buffer saline. The spectrum peak point graph of a Acyclovir (absorbance versus wave length) was determined. The Acyclovir shows the absorbance maxima at 252.0 nm in 7.4 pH Phosphate buffer saline.

Preparation of calibration curve of Acyclovir Procedure

10 mg of Acyclovir was accurately weighed and transferred to a 10 ml volumetric flask containing 10 ml of 7.4 pH Phosphate buffer saline and shaken to dissolve. The solution resulted is ≈1000 μg/ml. Then 0.1 ml of this solution is transferred to another 10ml volumetric flask to obtain solution of 10 μg/ml dilutions were done. The absorbance was taken on double beam U.V. spectrophotometer using absorbance maxima at 252 nm. The absorbance values were plotted against concentration (μg/ml) to obtain the standard calibration curve.

Formulation Development

Ethosomes has been formulated by the following method:-

Cold method

Acyclovir entrapped ethosomal vesicles were prepared by "Cold Method" with small modification. To prepare ethosomes, soya lecithin phosphotidylcholine (Phospholipid) was dissolved in ethanol in covered vessel with forceful stirring at room temperature. Added propylene glycol at 40ºC to it during stirring. Mix up to 30ºC. The drug is then dissolved in water and added dropwise gradually to phospholipid dispersion at 30ºC on magnetic stirrer at 1700 rpm in a closed vessel and mixing was uninterrupted further for half an hour. The ethosomal suspensions prepared were subjected to sonication using ultrasonic bath for an hour with a cycle of 10 mins. Nanosized ethosomes were impulsively produced with this method. The preparation was stored at 4ºC overnight. The composition of various ethosomal vesicles is given below.

Table 1: Composition of Ethosomes

rmulation Code	hospholipid (% w/v)	Ethanol (% w/v)	ropylene glycol (% w/v)	Distilled Water (up to % w/v)	Observation
F1	0.25	2	5	25	Vesicles not formed
F2	0.50	2	5	25	Vesicles not formed
F3	0.75	2	5	25	Vesicles not formed
F4	1.0	2	5	25	Vesicle not formed
F5	0.25	5	5	25	Vesicles formed
F6	0.50	5	5	25	Vesicles not formed
F7	0.75	5	5	25	Vesicle not formed
F8	1.0	5	5	25	Vesicle not formed
F9	0.25	10	5	25	Vesicle not formed
F10	0.50	10	5	25	Vesicle not formed
F11	0.75	10	5	25	Vesicle not formed
F12	1.0	10	5	25	Vesicles not formed

Optimization of Blank Ethosomes

Table 2: Final optimized formulation of drug loaded Ethosomes

S.no.	Formulation code	Drug (mg)	Phospho-lipd (% w/v)	Ethanol (% w/v)	Propylene Glycol (% w/v)	Distilled Water (up to) (% w/v)
1.	F5	10	0.25	5	5	25

Characterization Of Ethosomal Formulations

Optical Microscopy Observation Procedure

The ethosomal dispersion was applied on the glass slide with a glass rod. Formation of multilamellar vesicles was established by investigating the ethosomal suspension under an optical microscope with the magnification power of 100X. Photographs of vesicles was taken using olympus camera.

Surface morphology and Vesicle shape of ethosomal vesicles

Surface morphology and the vesicle shape were to be characterized by Transmission electron microscopy.

Procedure

One drop of formulation was positioned on a clear-glass slide, then it was air-dried and covered with gold by Sputter coater. Afterward it was visualized under scanning electron microscope (Jeol 5400, Japan).

Vesicle size and size distribution

The size of the final ethosomal formulation were measured by Particle size analyzer (Malvern mastersizer) Malvern Instrument Ltd., Model S., Ver.2.15, Malvern, UK.

Zeta Potential Determination

Zeta potential determination is generally performed to determine the surface charge of the drug loaded vesicle Surface charge of drug-loaded vesicles was determined using Zetasizer. Analysis time was kept 60 sec, and average zeta potential of the optimized vesicles was determined.

Formulation Development Of Ethosome Loaded Gel For Trandermal Delivery

Method of preparation

Computed exact quantity of Methyl Paraben, Glycerine and weighed amount of Propylene Glycol. Dissolved calculated number of Methyl Paraben, Glycerin and weighed amount of Propylene Glycol in about 35 ml beaker and stirred at high speed with mechanical stirrer or (sonicator). Then carbopol 943 was added gradually to the beaker and stirred. Neutralized the solution by leisurely adding triethanol solution with stirring until gel is formed. Ethosomes (5%) of optimized formulation integrated into the gel to form ethosomes loaded gel for topical delivery.

Table 3: Composition used for the hydrogel formulations

Ingredients (gm)	HG1	HG2	HG3	HG4	HG5	H6
Ethosomes(gm) (equivalent to 10 mg of ACV)	2.5	2.5	2.5	2.5	2.5	2.5
Carbopol 940(gm)	0.25	0.30	0.35	0.40	0.45	0.5
Propylene Glycol(gm)	10	10	10	10	10	10
Methyl Paraben(gm)	0.08	0.08	0.08	0.08	0.08	0.08
Triethanolamine (ml)	1.2	1.2	1.2	1.2	1.2	1.2
Glycerin(ml)	5	5	5	5	5	5
Ethanol(ml)	10	10	10	10	10	10
Distilled Water(ml) (q.s)	50	50	50	50	50	50

*where HG means hydrogel formulations and ACV means Acyclovir.

Evaluation Of Topical Gels

Appearance and consistency

The physical appearance was visually observed for the consistency of topical gel formulations.

Washability

Formulations were applied on the skin and then effortlessness and degree of washing with water.

Extrudability determination of formulations

The hydrogel gel formulations were packed into collapsible metal tubes. Weight were putted over collapsible tube in ascending order .At particular weight hydrogel extrude out. The extrudability of the formulation was checked.

Determination of Spreadability

An important criterion for topical gels is that it must possess good spreadability. Spreadability is a term expressed to denote the extent of area to which 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 the time in seconds taken by the two slides to slip off from a formulation, placed between, the two slides under the application of a certain load. Lesser the time taken for the separation of two slides, better the spreadability of the gel.

Determination of pH

The pH of the topical gels is to be resolute by digital pH meter. One gram of gel was dissolved in 25 ml of distilled water and the electrodes were then dipped in to the gel formulation until steady analysis obtained. And even interpretation was noted. The measurements of pH of every formulation were replicated two times.

Viscosity

The measurement of viscosity of the prepared gel was done by means of Brookfield digital Viscometer. The viscosity was calculated using spindle no. 6 at 10 rpm and 25^{0}C. The satisfactory quantity of gel was packed in appropriate wide mouth container. The gel was filled in the large mouth container in such technique that it should sufficiently allow to immerse the spindle of the Viscometer.

Samples of the gels were permissible to settle over 30 min at the stable temperature (25 ±1^{0}C) prior to the measurements.

Drug content

The drug content was determined by taking 1 g of gel (equivalent to 10 mg of Acyclovir) in 10 ml volumetric flask diluted with Phosphate buffer 7.4 pH. The on top of solution was properly diluted and determined using UV – Visible spectrophotometer at 252nm.

In-vitro Drug Release Studies Using the Prehydrated Cellophane Membrane

Preparation of cellophane membrane for the diffusion studies:

The cellophane membrane (25 cm x 2cm) was in use and washed in the running water. It was then drenched in distilled water for 24 hours, prior to used for diffusion studies to take away glycerin present on it and was mounted on the diffusion cell for additional studies.

Diffusion Studies

The in-vitro diffusion of drug from the different gel preparations was calculated using the traditional standard cylindrical tube fabricated in the laboratory; a simple modification of the cell is a glass tube of 15mm internal diameter and 100mm height. The dispersal cell membrane was useful with one gram of the formulation and was attached firmly to one end of the tube, the other end reserved open to ambient conditions which acted as donor compartment. The cell was reversed and wrapped up somewhat in 250 ml of beaker containing neutralizing Phosphate buffer, recently organized (7.4 pH as a receptor base and the system was maintained for 2 hrs at 37± 0.5° C. The media was stimulated using magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn periodically at programmed time interval of 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 min and replaced by an equivalent volume of the receptor medium. The aliquots were correctly diluted with the receptor medium and analyzed by UV-Visible spectrophotometer at 252 nm by means of neutralizing Phosphate buffer 7.4 pH as blank.

RESULTS AND DISCUSSION

Identification test by FTIR

The identification study of Acyclovir is done by the FT-IR spectroscopy with respect to marker

IR Spectrum of Acyclovir

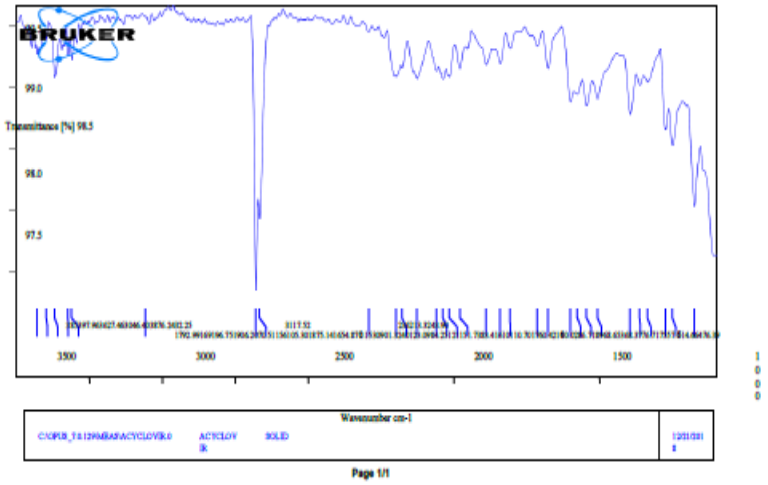


Fig.1: FT-IR Spectrum of Drug sample (Acyclovir)

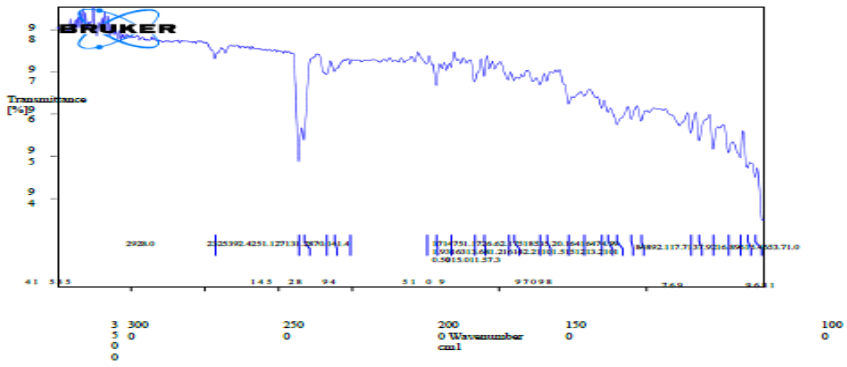


Fig. 2: FT-IR Spectrum of Reference (Acyclovir)

Result

The IR spectra shows N-H stretching at 3400-3250 cm^{-1}, N-H bending 1650-1580, C- H aromatic starching 3100-3000 cm^{-1}, C-H aliphatic stretching 3000-2850 cm^{-1}, and C=O bending 17600-1665 cm^{-1}, which are characteristics of acyclovir.

Table 4: preformulation

S.No.	Parameter	Observation
1.	Solubility	Insoluble in Ethanol, Insoluble in Methanol, and soluble in water and soluble in buffer pH 7.4
2.	Loss on drying	0.0285%
3.	pH (1%w/v solution in water)	10.5
4	Moisture content with KF	2.08 mg
5	Melting point determination	256^{0}C
6	Untapped Density	0.50 g/cc
7	Tapped Density	0.83 g/cc
8	Compressibility index	39.75%.
9	Hausner ration	1.66.
10	Angle of repose	54.5^0

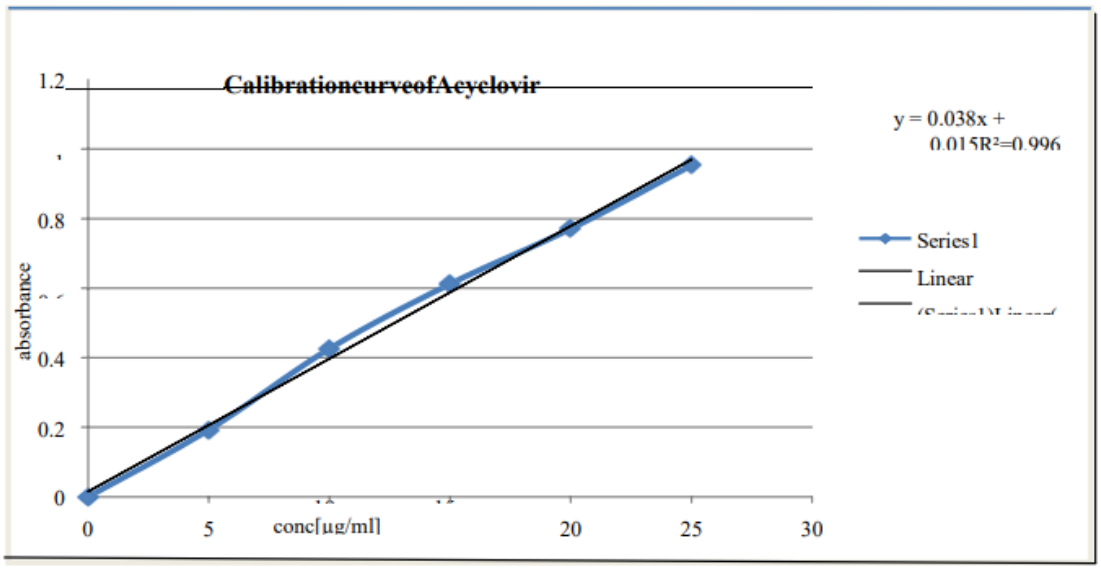


Fig.3: Calibration curve of Acyclovir

Result

The calibration curve was plotted between concentration and absorbance. It gives the straight line in the concentration range between

5- 25µg/ml. The correlation coefficient was found to be 0.996.This shows that it follows Beer"s law.

Characterization Of Ethosomal Formulations

Optical Microscopy Observation

The ethosmal dispersion is spread on a glass slide with the help of glass rod. A development of unilamellar vesicles was confirmed by examining the ethosomal dispersion under an optical microscope with the magnification power of 1000 x. (Olympus CX41, Philippines) Photographs of vesicles is taken using Olympus camera.

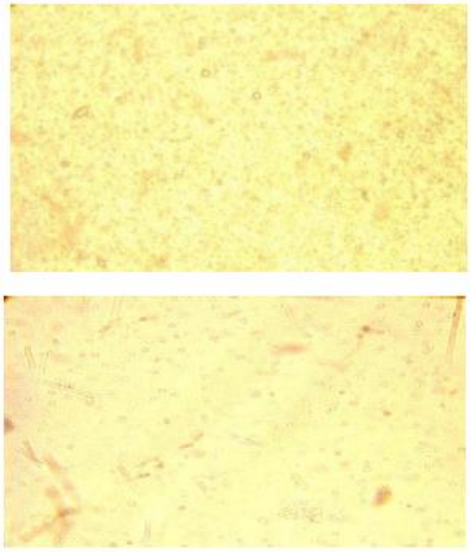


Fig.4: Photographs of vesicles of optimized Formulation

Result

The optical microscopy was performed and the preparation was viewed under 1000x magnification. The spherical shaped vesicle were observed. This confirms that the formulation has ethosomes.

Vesicle size and size distribution

Table 5: Vesicle size and size distribution

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	322.8 nm	93.0 nm	296.4 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	322.8 nm	93.0 nm	296.4 nm

Cumulant Operations

Z-Average : 499.5 nm

PI : 0.345

Result

The particle size analysis of ethosomes was done and the size was determined as 499.5 nm and PI was found to be 0.345. Which was good

Zeta Potential Determination

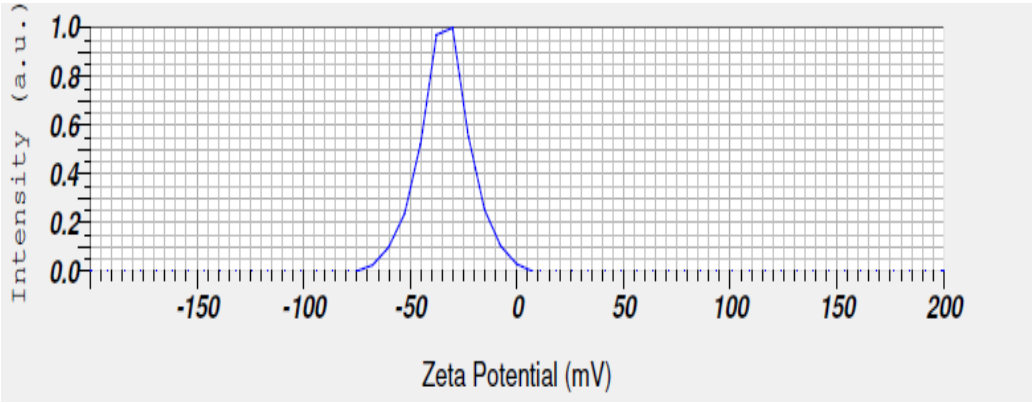
. **Table 6: Result of zeta potential determination**

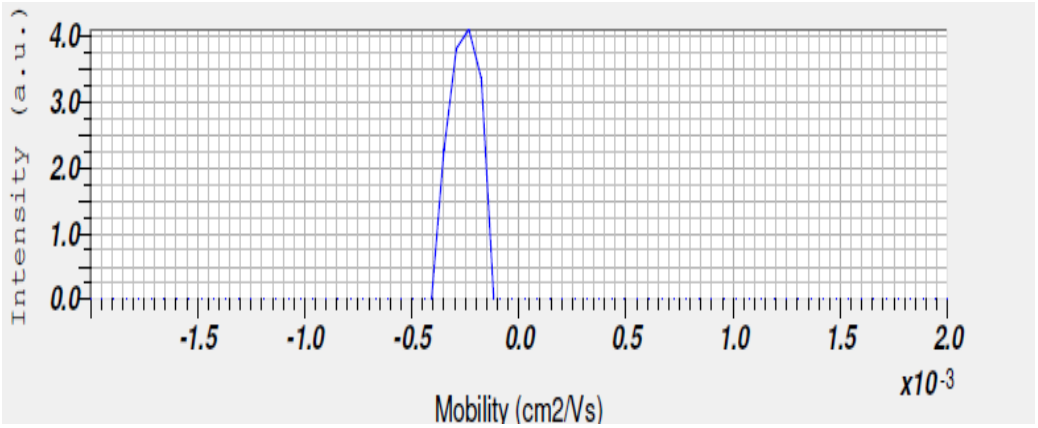
Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-33.6 mV	-0.000260 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean) : -33.6 mV

Electrophoretic Mobility Mean : -0.000260 cm^2/Vs





Result

The zeta potential of Acyclovir containing ethosomes was found to be -33.6 mV. Which was good.

Evaluation Of Ethosomal Gel

Evaluation of gel formulation

Result of Physiorheological Characteristic

Table 7: Result for the physiorheological property of gel

Formulation	Colour	Clogging	Homogeneity	Texture
HG1	Clear	Absent	Good	Smooth
HG 2	Clear	Absent	Good	Smooth
HG 3	Clear	Absent	Good	Smooth
HG 4	Clear	Absent	Good	Smooth
HG 5	Clear	Absent	Good	Smooth
HG 6	Clear	Absent	Good	Smooth

Result

In the above formulations of gels, it has been noted that all of them has clear colour,no clogging, good homogeneity and smooth texture.

Results of washability, Extrudability and spreadability.

Table 8: Result for the washability and extrudabliity and Spreadability for gels

Formulation	Washability	Extrudability	Spreadability(gcm/sec)
HG1	Good	Average	13.33 ±0.32
HG 2	Good	Average	13.06± 0.64
HG 3	Good	Average	13.07± 0.54
HG 4	Good	Average	13.05± 0.95
HG 5	Good	Average	12.00±0.15
HG 6	Good	Average	12.54± 0.20

Result

In the above formulations of gels, they have good washability ,extrudability.and spredability.

Results of the pH,Viscosity and Percentage drug content.

Table 9: Results for the pH, Viscosity and Percentage drug content of different formulations for gel

Formulation	pH	Viscosity (cps)	Percentage drug content
HG1	6.9± 0.15	2500	87.97 ± 0.52
HG 2	7.2± 0.20	2700	85.96 ± 0.95
HG 3	6.8± 0.5	3000	89.92 ± 0.69
HG4	7.1± 0.31	3200	85.92 ± 0.53
HG5	6.9± 0.41	3500	98.95 ± 0.63
HG 6	7.2± 0.52	4500	89.97 ± 0.52

Result

The above formulations of topical gels have different pH value for different formulations. In the above formulations of gel the spreadibility of the HG 5 was good. The formulation HG 5 has good viscosity. In the above formulations of different gel the percentage drug entrapment was found that HG 5 has maximum percentage of drug content.

In-vitro Drug release of HG 5 formulation

Table 10: In-vitro drug release of the optimized formulation(HG-5) of topical gel

S.No.	Time (in hrs.)	Absorbance	Percentage Drug release	Correction factor	Percentage cumulative drug release
1	0.5	0.121	28.4038	1.70423	28.4038
2	1	0.155	36.385	2.1831	38.0892
3	1.5	0.201	47.1831	2.83099	51.0704
4	2	0.255	59.8592	3.59155	66.5775
5	4	0.289	67.8404	4.07042	78.1502
6	6	0.355	83.3333	5.0000	94.8826

Result

The in-vitro drug release of optimized formulation of the topical gel. The percentage cumulative drug release was found to be 94.8826.

Release kinetics of optimized formulation (HG 5) Zero order release Kinetics

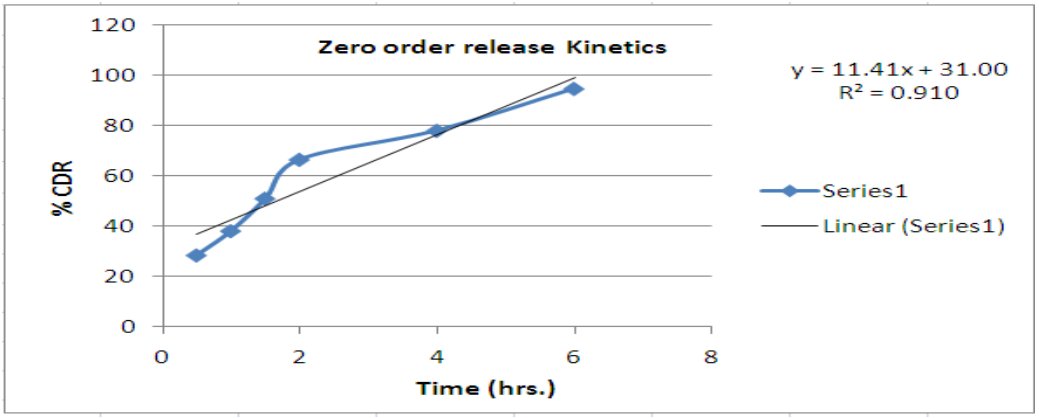


Fig. 5: Zero order release kinetics for HG-5 formulation

First Order release kinetics of optimized formulation (HG 5)

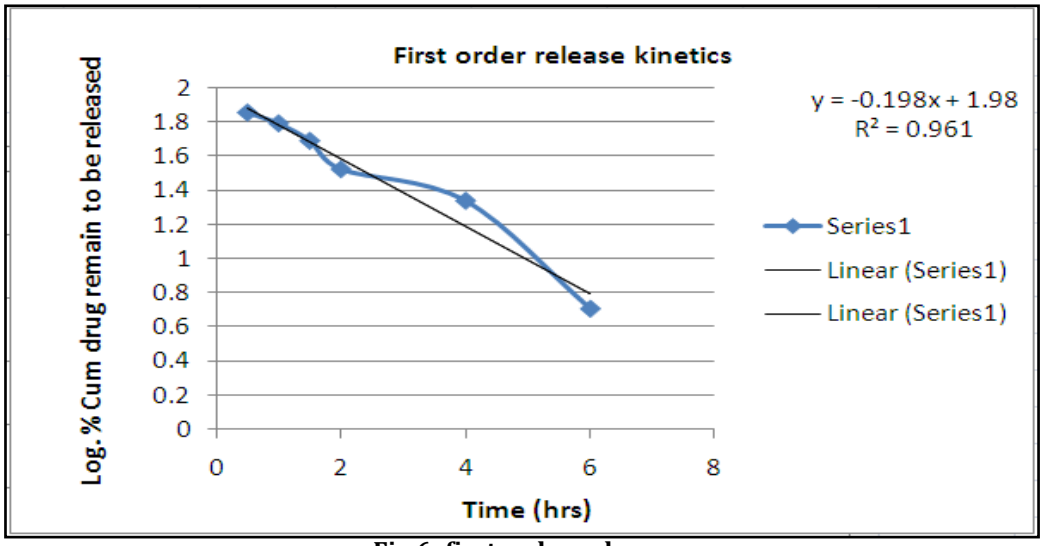


Fig.6: first order release

Higuchi release kinetics of optimized formulation (HG 5)

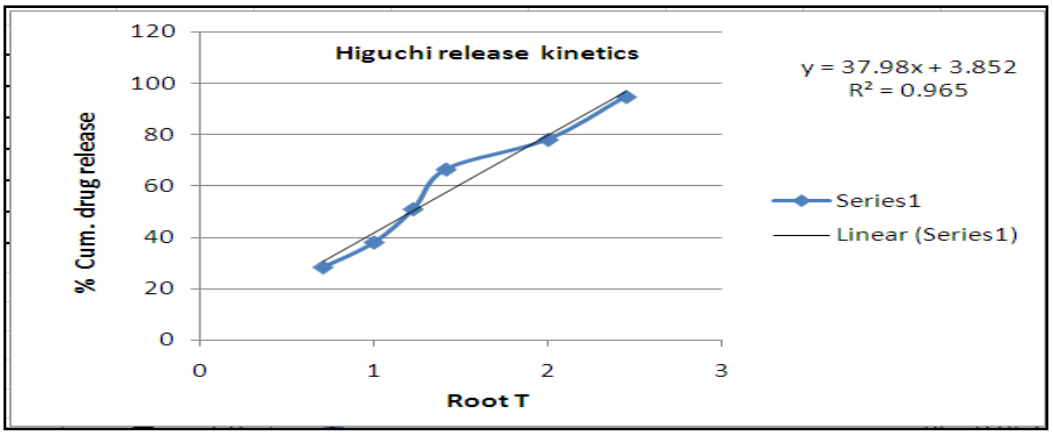


Fig.7: Higuchi release kinetics for HF5 formulation

Kinetics data of optimized formulation (HG 5)

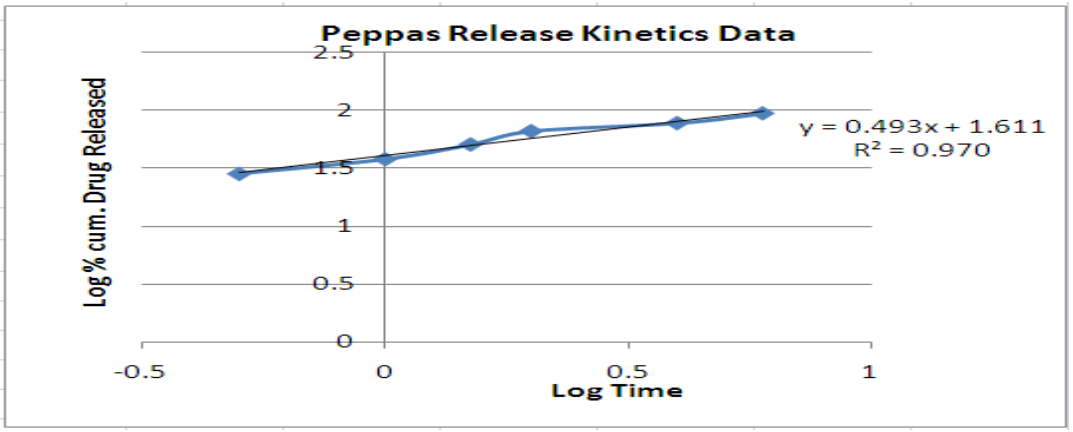


Fig 8: peppas release kinetics for HG 5 formulation

In the present study, the optimized formulation as prepared by the cold method and evaluated for the physiorheological properties such as colour, clogging, homogeneity, texture, washability, extrudability, spreadability, pH, viscosity, percentage drug content and in-vitrodrug release. The result obtained indicates that the optimized formulation was subjected to fit the test by linear regression analysis according to Zero order, first order kinetics equations, Higuchi and Korsmeyer"s peppas model in order to determine the mechanism of drug release. When the regression coefficient value was compared, it was observed that „r" value of Korsmeyer"s peppas was maximum that is 0.970. Hence indicating drug release from formulation was found to be following Korsmeyer's peppas kinetics.

CONCLUSION

It is to be concluded from the results of the study that hydrogel formulation is a potentially vehicle for topical delivery of Acyclovir. Soft malleable vesicles consisting of phospholipids and higher concentration of ethanol exhibited synergistic effect of phospholipids and ethanol on permeation providing elastic liposomes (ethosomes) are better carriers for acyclovir topical delivery. Vesicles with appropriate size and reasonable entrapment efficiency can be prepared. Results of the in-vitro release study through the skin revealed higher transdermal flux and hydrogel formulation with cold method in comparison to hot method.The present study demonstrated that with the help of topical drug delivery of ethosomal system, acyclovir can be successfully delivered through skin for the treatment of herpes simplex virus infection. Acyclovir hydrogel delivery is capable of prolonging drug release that might reduce the dosage frequency. Further, these results and finding may prove helpful for development and scaling up a new formulation.

REFERENCES

1. Kumar P, Sankar C and Mishra B. Delivery of macromolecules through skin. The Indian Pharmacist 2004, 7-17.
2. Rizwan M, Aqil M, Talegoankar S, Azeem A, Sultana Y and Ali A, Enhanced transdermal drug delivery techniques: an extensive review Delivery of macromolecules through skin, The Indian Pharmacist, 2004, 7-17.
3. Kumar R and Philip A, Modified Transdermal Technologies: Breaking the Barriers of Drug Permeation via the Skin, Trop J Pharm Res. 2007, 6(1):633-644.
4. Jain S, Bhandra D, Jain S and Jain N K, Transfersomes- A Novel carrier foreffective transdermal drug delivery controlled and novel drug delivery 1stEdition, CBS Publishers and Distributors New Delhi 1997: 426-451.
5. Jain N, Talegonkar S and Jain N K, New ways to enter the blood stream:Emerging strategies in transdermal drug delivery, The Pharma Review.Sep-Oct 2004,1- 60.
6. Kumar P, Sankar C and Mishra B, Delivery of macromolecules through skin. The indianPharmacist 2004, 7-17.
7. Touitou E., Dayan N., Bergelson L., Godin B and Eliaz M.. Ethosomes-novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Control. Rel. 2000, 65, 403-418.
8. Manosroi A., Jantrawut P., Khositsuntiwong N., Manosroi W. and Manosroi J., Novel Elastic nanovesicles for Cosmeceutical and Pharmaceutical Applications. Chiang Mai. J. Sci. 2009; 36(2), 168-178.
9. Maestrelli F., Capasso G., Maria L., Rodríguez G., Rabasco A.M., Ghelardini C. and Mura P., Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. J. Lipo.Resear. 2009, 1-8.
10. Bhalaria M.K., Naik S. and Mishra A.N., Ethosomes: A novel delivery system for antifungal drug in the treatment of topical fungal diseases. Indian J. Exp. Biology. 2009; 47, 368-375.
11. Verma D.D. and Fahr A., Synergistic penetration effect of ethanol and phospholipids on the topical delivery of Cyclosporin A. J. Control Rel. 2004; 97, 55-66.
12. Sultana SS,Sailja A, Ethosomes: A Novel approach in the design of transdermal drug delivery system,International Journal of MediPharm Research,Vol.02, , pp 17-22, 2015
13. Touitou E., Compositions for applying active substances to or through the skin. US Patent 1995 538, 934. Jain S, Umamaheshwari R B, Bhadra D and Jain N K., Ethosomes: A novel vesicular carrier for enhanced transdermal delivery of an anti HIV agent. Indian J. Pharm Sci 2004; 66(1), 72- 81.
14. Tauitou E, Dayan M, Bergelson L, Godin B and Eliaz M., Ethosomes- novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J Con Release 2000, 65, 403- 413.