Formulation and Evaluation of Acyclovir Hydrogel for Topical Delivery

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Abstract

This research encompasses formulation and evaluation of acyclovir hydrogel for topical delivery. 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.

Keywords: *hydrogel formulation, ethosomes, in-vitro release, Herpes simplex virus, Acyclovir, Anti-viral*

1. Introduction

The human skin is an easily available surface for drug delivery. Over past three decades, developing controlled drug delivery has become even more important in the pharmaceutical industry. Skin of the normal adult body covers a surface of about 2mm and receives about one- third of the blood circulating through the body.The human skin surface is known to contain, on an average, 10- 70 follicles and 200-250 sweat glands on every square centimeter of the skin area[1]. Transdermal drug delivery- the transport of drugs across the skin and into systemic circulation is discrete from topical drug penetration which targets restricted areas. Transdermal drug delivery takes advantage of relative accessibility of the skin[2].Transdermal route presents several potential advantages more than conventional routes like avoidance of first pass metabolism, inevitable and extended duration of activity, minimizing undesirable side effects, usefulness of short half-life drugs, improving physiological and pharmacological response.

Ethosomes are the tailored form of liposomes that are more in ethanol content. This ethanol fluidized both ethosomal lipids and bilayers of the stratum corneum intercellular lipid[3].The vesicles have been well recognized for their importance in cellular communication and particle transportation for several years.The use of lipid vesicles, such as liposomes, as reliable vehicles for topical drug delivery system has fascinated increasingly attention due to their efficiency in entrapment of drug and delivering them to the skin[4].

Herpes simplex is a viral disease from the herpes viridae family caused by both Herpes simplex virus type 1(HSV -1) and type 2 (HSV-2). Infection with the herpes virus is categorized into one of several distinct disorder based on the site of infection [5]. Acyclovir ointment and creams are available which are used for local applications. These have limited drug penetrability.So, to enhance the penetrability and reduce the frequency of drug administration hydrogel (ethosomal gel) has been prepared.Combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper penetration and distribution in the skin lipid bilayers. Flexibility of ethosome membrane minimizes the risk of complete vesicle rupture in the skin. The ethosomes are useful carrier for the acyclovir as they help in improving topical as well as systemic availability of the drug as they can penetrate in deeper layers and side effects are also reduced and also provides sustained release [6].

The main aim of this work is to study the usefulness of hydrogel for topical delivery of Acyclovir in the treatment of infections caused by Herpes simplex virus [7]. The topical delivery of hydrogel offers many advantages over oral systems that include improving the systemic bioavailability of drug by avoiding first pass metabolism and thus providing a sustained, controlled drug delivery.

2. Materials and Methods

2.1. Ethosomes has been formulated by the 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 [8].

2.2 Final optimized formulation of drug loaded Ethosomes

Table No.1:Final optimized formulation of drug loaded Ethosomes

Sr.no	Formulation co	Drug (r	Phospho-li (% w/v)	Ethanol (% w/v)	Propylene Glycol (% w/v)	Distilled Wa (up to) (% w/v)
1.	F5	10	0.25	5	5	25

2.3 CHARACTERIZATION OF ETHOSOMAL FORMULATIONS

2.3.1. Optical Microscopy Observation

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

2.3.3. The size of the final ethosomal formulation were measured by Particle size analyzer

2.3.4. 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 Zeta sizer.

2.4 FORMULATION DEVELOPMENT OF ETHOSOME LOADED GEL FOR TRANDERMAL DELIVERY

2.4.1 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 [9].

Table 2. Composition used for the hydrogel formulations

Ingredients (gm)	HG1	HG2	HG3	HG4	HG5	HG6
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

2.5 EVALUATION OF TOPICAL GELS

2.5.1 Appearance and consistency: The physical appearance was visually observed for the consistency of topical gel formulations.

2.5.2 Washability: Formulations were applied on the skin and then effortlessness and degree of washing with water**.**

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

2.5.4 Determination of Spreadability: 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.

2.5.5 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.

2.5.6 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.

2.5.7 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.

2.5.8 In-vitro Drug Release Studies Using the Pre hydrated Cellophane Membrane

2.5.8.1 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 use for diffusion studies to take away glycerin present on it and was mounted on the diffusion cell for additional studies.

2.5.8.2 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 [10]. 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

The data obtained was graphed as follows: -

- Cumulative % drug diffusion Vs time in min
- Log of cumulative % drug remain Vs time
- Cumulative % drug diffusion Vs square root of time
- Log of cumulative % drug diffusion Vs log time.

3. RESULTS AND DISCUSSION

3.1 PHYSICO-CHEMICAL PROPERTIES OF ACYCLOVIR

1) Colour: White crystalline powder

2) Odour: Characteristic

Table 3. Solubility of Acyclovir in different solvents

Sr. No	Solvent	Solubility
1	Ethanol	Insoluble
2	Chloroform	Soluble
3	Methanol	Insoluble
4	0.1 N HCl	Soluble
5	PBS (pH 7.4)	Soluble
6	Water	Slightly Soluble

3.2. Identification test by FTIR:

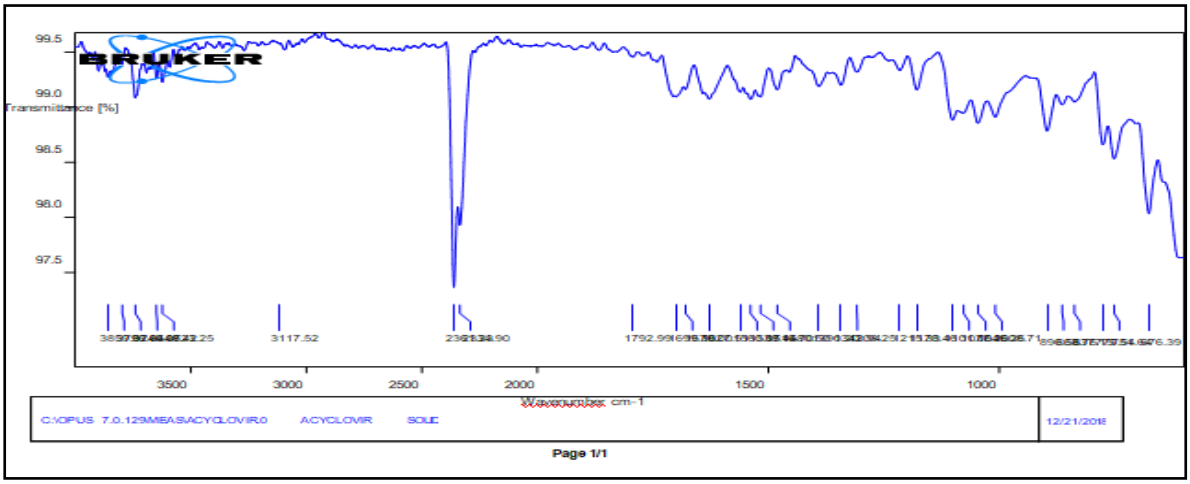


Figure.1 FT-IR Spectrum of Drug sample (Acyclovir)

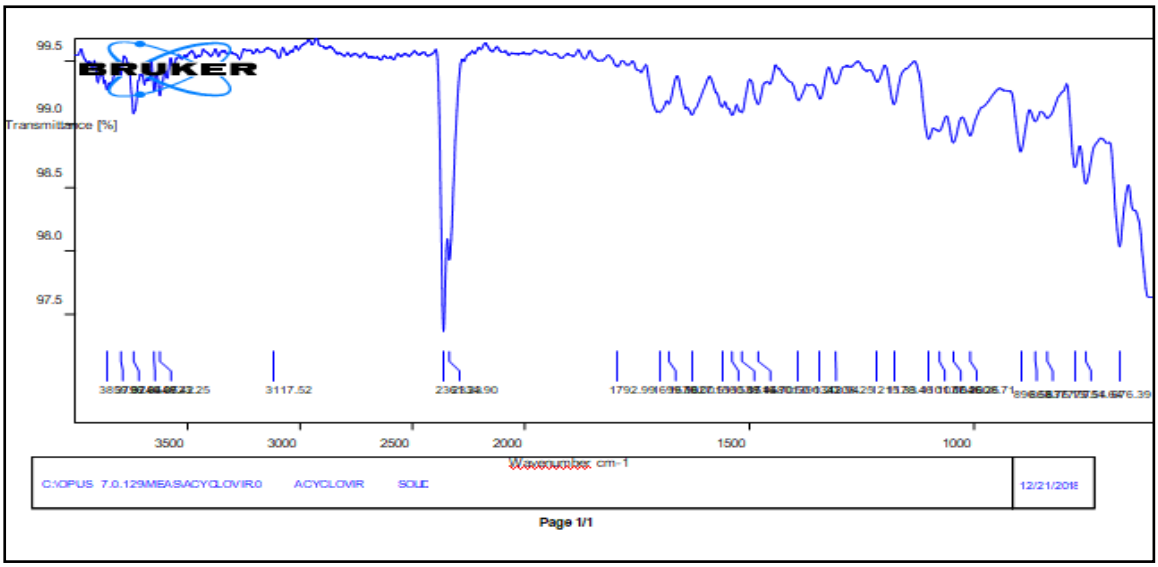


Figure.2 FT-IR Spectrum of Reference (Acyclovir)

Table 4. IR interpretation for Acyclovir

S.No.	Frequency, cm^{-1}	Functional group	Band
1)	3400–3250 cm-1	Primary,secondary amines, amides	N–H stretch
2)	1650–1580 cm-1	primary amines	N–H bend
3)	3100–3000 cm-1	Aromatic	C–H stretch
4)	3000–2850 cm-1	Alkanes	C–H stretch
5)	17600–1665cm-1	Carboxylic Acid	C=O bend

Table 5. Comparative data of preformulation

S.No	Parameter	Observation
1.	**Solubility**	Insoluble in Ethanol, Insoluble in Metha and soluble in water and soluble in bu 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**	256^0C
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

3.3 DETERMINATION OF λ_{max} BY UV-VISIBLE SPECTROSCOPY

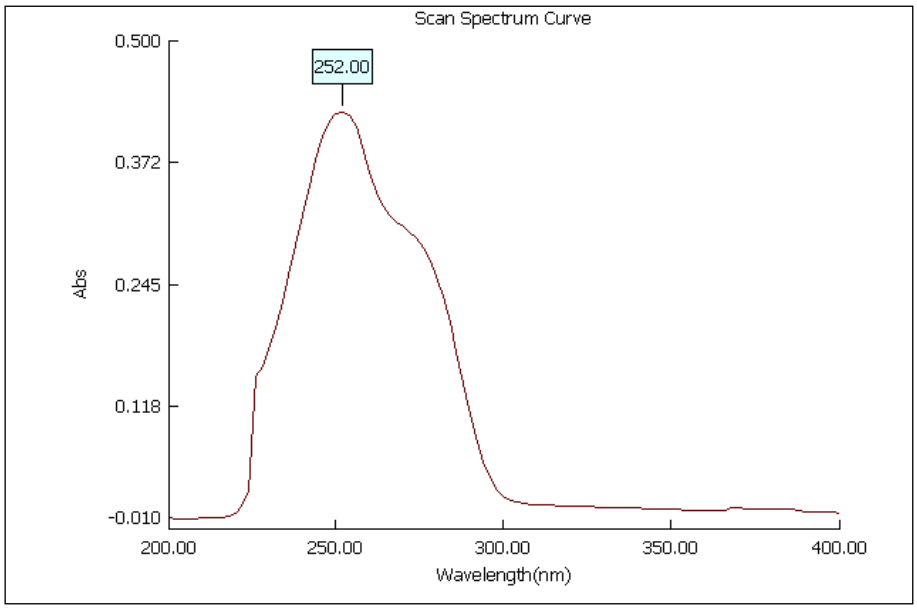


Figure 3. Determination of λmax of Acyclovir

Table 6.Calibration curve data of Acyclovir in 7.4 pH Phoshate Buffer at 252nm

S.No	Concentration (µg/ml)	Mean Absorbance at 252nm
1	0	0
2	5	0.193
3	10	0.426
4	15	0.612
5	20	0.771
6	25	0.953

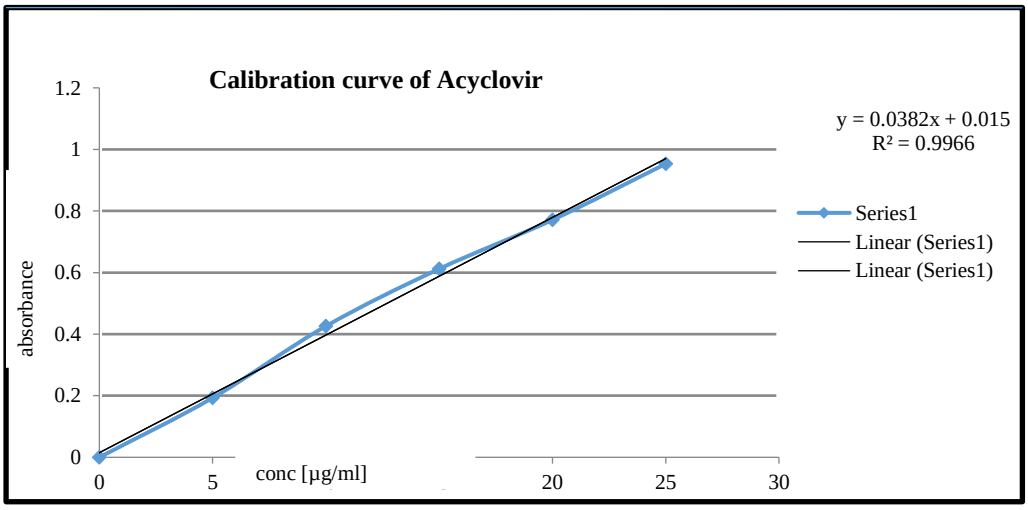


Figure 4. Calibration curve of Acyclovir

3.4 CHARACTERIZATION OF ETHOSOMAL FORMULATIONS

3.4.1 Optical Microscopy Observation

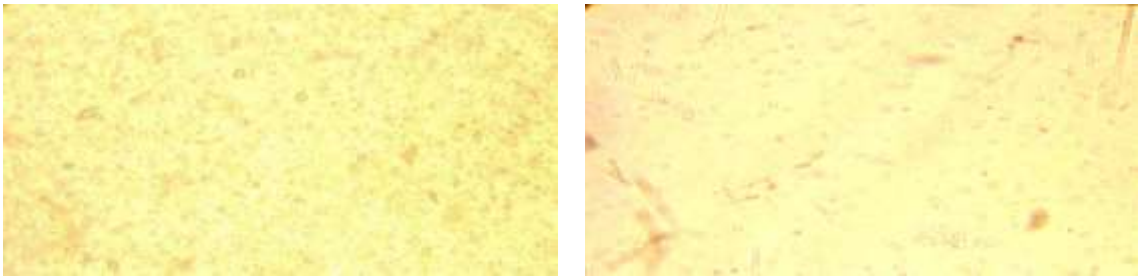


Figure 5. Photographs of vesicles of optimized Formulation

3.4.2 Vesicle size and size distribution

Table 7. 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

3.4.3 Zeta Potential Determination

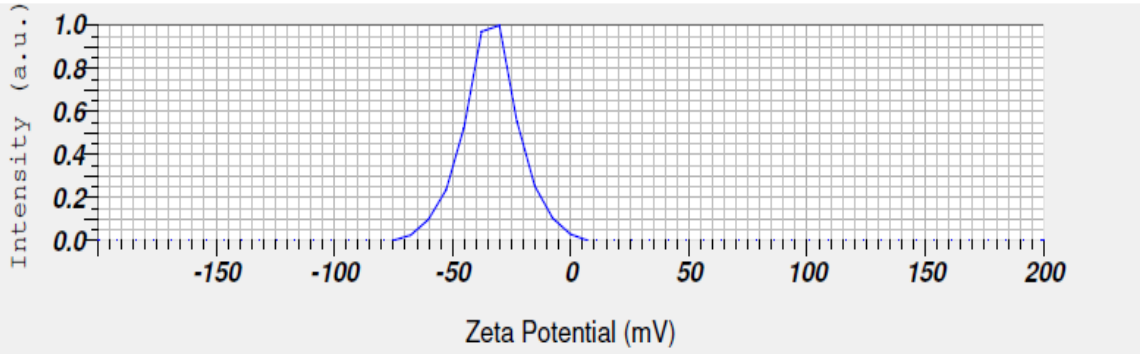
.Table 8. Result of zeta potential determination

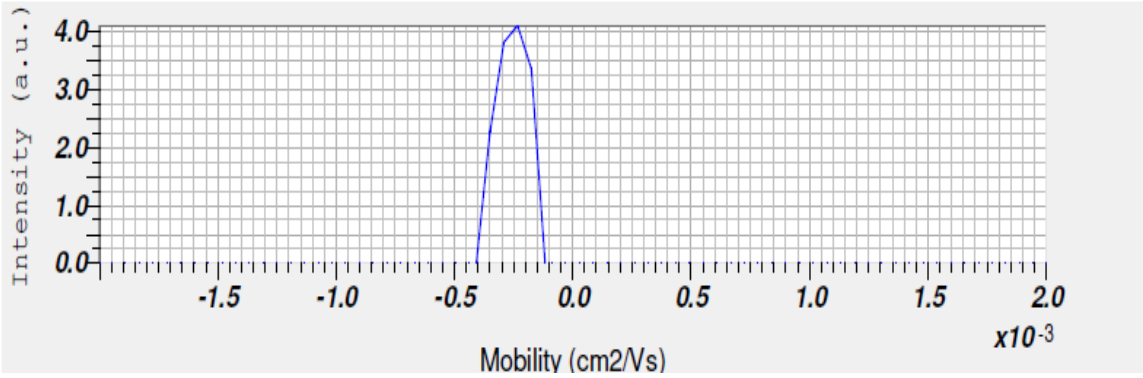
Calculation Results

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.

3.5 EVALUATION OF ETHOSOMAL GEL

3.5.1 Result of Physiorheological Characteristic

Table 9. 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

3.5.2 Results of washability, Extrudability and spreadability

Table 10. Result for the washability and extrudabliityand Spreadabilityfor 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

3.5.3 Results of the pH, Viscosity and Percentage drug content

Table 11. Results for the pH,Viscosity and Percentage drug contentof different formulations for gel

Formulation	pH	Viscosity (cps)	Percentage drug content
HG1	10.3 ± 0.15	2500	87.97 ± 0.52
HG 2	10.5 ± 0.20	2700	85.96 ± 0.95
HG 3	11.0 ± 0.5	3000	89.92 ± 0.69
HG4	11.1 ± 0.31	3200	85.92 ± 0.53
HG5	10.7 ± 0.41	3500	98.95 ± 0.63
HG 6	10.8 ± 0.52	4500	89.97 ± 0.52

3.5.4 *In vitro* Drug release of HG 5 formulation

Table 12. *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 d 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

3.6 Release kinetics of optimized formulation (HG 5)

3.6.1 Zero order release Kinetics

Table 13. Zero order release kinetics of HG 5 formulation

S. No.	Time (in hrs.)	Percentage cumulative drug release
1	0.5	28.4038
2	1	38.0892
3	1.5	51.0704
4	2	66.5775
5	4	78.1502
6	6	94.8826

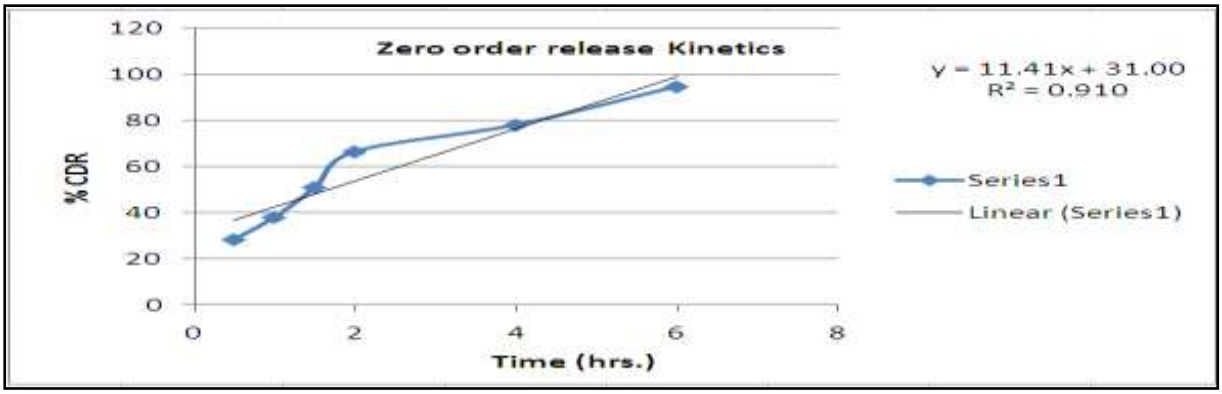


Figure 6. Zero order release kinetics for HG-5 formulation

3.6.2 First Order release kinetics of optimized formulation (HG 5)

Table 14. first order release kinetics of optimized formulation

Sr. No.	Time (in hrs.)	Log. Percentage cumulative Drug remain be released
1	0.5	1.85489
2	1	1.791766
3	1.5	1.689572
4	2	1.524039
5	4	1.339447
6	6	0.709049

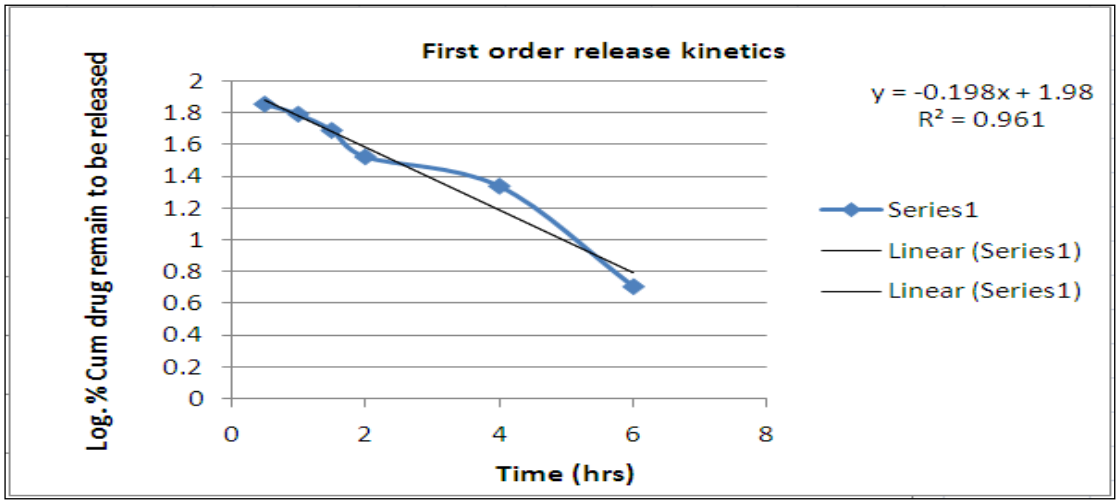


Figure7. first order release kinetics for HG-5 formulation

3.6.3 Higuchi release kinetics of optimized formulation (HG 5)

Table 15. Higuchi kinetics of optimized formulation

Sr. No.	Root T	Percentage cumulative Drug rele
1	0.707107	28.4038
2	1	38.0892
3	1.224745	51.0704
4	1.414214	66.5775
5	2	78.1502
6	2.44949	94.8826

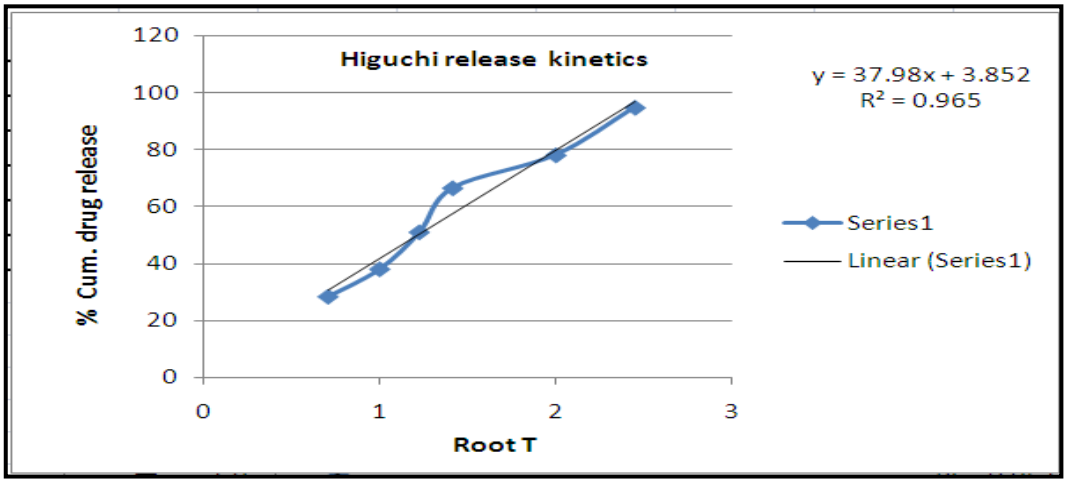


Figure 8. Higuchi release kinetics for HG5 formulation

3.6.4 kinetics data of optimized formulation (HG 5)

Table 16. Peppas release kinetic data of optimized formulation

S. No.	Log Time	Log percentage Cumulative Drug relea
1	-0.30103	1.453376
2	0	1.580802
3	0.176091	1.708169
4	0.30103	1.823327
5	0.60206	1.89293
6	0.778151	1.977187

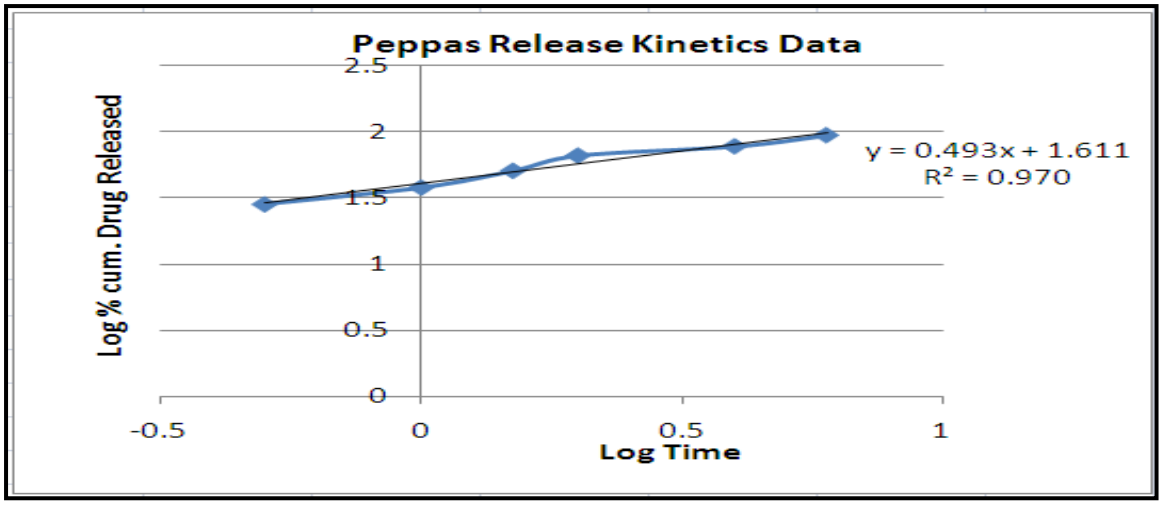


Figure 9. Peppas release kinetics for HG 5 formulation

4. Discussion

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-vitro drug 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**.

5. 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.

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