WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 11, 1425-1442. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF ACYCLOVIR LOADED MICROSOMAL GEL

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Article Received on
30 June 2021,

Revised on 21 July 2021,
Accepted on 10 August 2021

DOI: 10.20959/wjpps202111-21362

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ABSTRACT

Microsomes are novel drug delivery system that promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with skin that problems. Acyclovir is white crystalline, tasteless, odorless, substance was sparingly soluble in water. λ_{max} was determined in Phosphate buffer (pH 7.4) solvent at 251.3 nm. Standard calibration curve was prepared using concentration range 04-20µg/ml. Five different formulations were prepared all the sodium alginate solutions were prepared by dissolving the respective amounts in distilled water. Dried microcapsules were subjected for Evaluation were found yield between 83.54 to 94.22 %, mean particle size between 568.65 to 751.82 µm and % encapsulation between 80. 21 to 97.01% and swelling Index between 3.04to3.64, shape was spherical with slightly elongated tips found in some microspheres. On the basis of various parameter of evaluation of microsomal gel formulations, pH of all formulations were adjusted about 6.63, viscosity at 5 rpm was maximum at 58767 cps, % drug content between 98.69 to 100.35% and Spreadability maximum 12.69 gm.cm/sec**.** All formulation (P-1 to P-5) with a blank formulation (P-0 only pure drug) and a marketed formulation were subjected for diffusion study with cellophane membrane and egg membrane. Cumulative amount of drug released Q (µg/cm^2) at different time intervals and steady state flux (Jss, µg/cm^2.hr) were obtained. Also permeability coefficient (Kp) and enhancement ratio (ER) are calculated and found **P-5** has highest enhancement ratio (ER) 2.29 and **P-4** has 2.02. Also it was observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases. Accelerated Stability studies for 30 days was performed with optimized Microsomes batch **P-5** for physical examination observed no visible change, % Drug content after stability and before stability were 98.01 and 100.33 respectively.

KEYWORD: Acyclovir, Microsomal Gel, Microsome, Evaluation**.**

INTRODUCTION

Microsomes are novel drug delivery system that promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with skin that problems generally encountered in semisolid dosage forms. Microencapsulation is the process in which small droplets or particles of liquid or solid material are surrounded or coated by a continuous film of polymeric material.[1] Microencapsulation is for sustained or prolonged release of the drug. Microencapsulation technique the liquid drugs can be converted in a free flowing powder. Microencapsulation is widely used in the pharmaceutical and other sciences to mask tastes or odors, prolong release, impart stability to drug molecules, improve bioavailability, and as multi-particulate dosage forms to produce controlled or targeted drug delivery.[1-4] Novel drug delivery system which were initiate with the course of optimization the bioavailability by the modification of the bioavailability of the drug concentration in blood.[5,6] with the sustained and controlled release products, drug therapy can be improved that is the common goal achieved over with their non sustained and controlled release with the same drug. The controlled release dosage forms are so designed and formulated as having the sustained action, sustained release, prolonged action, delayed action and time release medication. [7] This has been done by developing the drug entities, discovering of new polymeric material that are susceptible for prolonged the drug release, safety, improvement in therapeutic efficacy.[8] Microencapsulation can be achieved by various methods e.g. air suspension, coaservation phase separation, spray drying, pan coating etc11.

MATERIAL AND METHODS

Acyclovir was obtained from Mylan laboratories limited, Nashik, Chitosan, Sodium alginate and Calcium chloride from RRL, Bhopal, Nerolidol from Sigma Aldrich, Mumbai, Methyl paraben, Propyl paraben and Carbapol from MCW Industries Indore. Aloe vera was purchased from Health Secure (India) Pvt. Ltd. All used solvents and chemicals were laboratory grade.

PREFORMULATION STUDY

Characterization of drug sample

Organoleptic properties: The drug sample was examined for its color, odour and appearance.

Melting point determination: The Melting point was determined by the capillary method using Digital Melting point apparatus.

Solubility: The solubility of Acyclovir was determined by adding excess amount of drug in the solvent at 37^0C and kept for 3 days for equilibrium in shaking incubator.

UV Spectroscopy

Determination of λ_{max} of Acyclovir in phosphate buffer

Accurately weighed 10 mg of acyclovir was dissolved in 10ml of solvent (1000 µg/ml) called A stock solution of the drug sample. was prepared in water and phosphate buffer of pH 7.4 by dissolving accurately weighed quantity of drug sample in 100 ml volumetric flask, it was then suitably diluted scanning was done in range of 200nm-400nm. The λ_{max} found was checked, whether it complies with the reported ones or not.

Calibration Curve of Acyclovir in phosphate buffer pH 7.4

Accurately weighed quantity of Acyclovir (10 mg) was dissolved in little quantity of phosphate buffer of pH 7.4 and volume was made up to 10ml (1000 µg/ml). Appropriate aliquots from above stock solution were and final volume was made up to 10 ml by phosphate buffer solution, so as to get solutions with drug concentrations of range 4 to 20 µg/ml. These solutions were then analyzed using UV spectrophotometer and a plot of absorbance v/s concentration was plotted.

Fourier Transform Infrared (FTIR) analysis

Acyclovir was dried in oven at 50^0C for 2 hour. The samples were prepared by mixed it thoroughly with potassium bromide. This physical mixture was compressed under pressure of 10ton/nm^2 & converted in a circular disc. This disc was then placed in the scanning slot of FTIR & scanned at range from 400 to 4000 cm^{-1} to obtain FTIR of spectrum with reference spectrum.

Formulation of Microsomes of Acyclovir

The microsomes of acyclovir were prepared according to the table. Briefly, all the sodium alginate solutions were prepared by dissolving the respective amounts in distilled water. To the sodium alginate solutions, 1% w/v of acyclovir was added under homogenization for 5 min to yield smooth dispersions of acyclovir in sodium alginate solutions. The chitosan solutions (1% w/v or 2% w/v) were prepared in 5% v/v aqueous acetic acid. To the chitosan solutions, respective amount of calcium chloride was added. The acyclovir–alginate

dispersions were taken into syringe fitted with 0.45 mm needle and dropped at the rate of 1ml/min into chitosan – calcium chloride solutions stirred at 100 RPM at room temperature to yield opalescent beads. The microspheres were allowed to harden for another 2 h, filtered, washed thrice with distilled water and kept for drying at 40 ºC in an oven for 24 h. After 24 h, the size of the beads reduced and they were kept in labeled self-sealing plastic bags.

Table 1: Composition of Microsomes of Acyclovir.

Code	Sodium alginate concentration (% w/v)	Calcium chloride concentration (% w/v)	Chitosan concentration (% w/v)
P1	2	5	1
P2	4	5	2
P3	4	2.5	1
P4	2	2.5	2
P5	3	2.5	1

Characterization of the ACV Microsomes

Calculation of % yield calculation

During this process, % yield was calculated as follows:

$$\% \text{ Yield } = \frac{\text{Practical yield}}{\text{Theoretical yield}} \text{ X } 100$$

Swelling Index

50 mg of microsomes were placed in water and set aside to swell over night. Microsomes were decanted using filter paper and weighed. The degree of swelling (α) was then calculated from the formula:

$$\text{Swelling Index } (\alpha) = \frac{\{\text{weight of swelled microsomes } (Wg) - \text{ Initial weight of microsomes } (Wo)\}}{\text{Initial weight of microsomes } (Wo)}$$

Particle Size Determination by Optical Microscopy

All the four batches prepared (P1 to P4) were analyzed for particle size by optical microscope. First the eye piece micrometer was calibrated using a stage micrometer and then on a clean glass slide a small quantity of microspheres were placed using a thin brush. Then they were covered carefully with a cover- slip and observed under 10X magnification. One hundred particles from each batch were counted and average particle diameter was found out by using the formula:

$$\text{Average particle diameter} = \sum n*d/ N$$

Where,

n = total no. of particles in that size range

d = Diameter of the particles of that size range

N = total no. of particles.

Determination of % Drug Content & Encapsulation Efficiency

20 mg of the microspheres from each batch were taken and digested in 100 ml of 0.1N HCl in a 100 ml volumetric flask and kept aside with intermittent shaking for 24 h. Then, the contents of the flask were filtered by using Whatman filter paper no.1. Then 1 ml of the filtrate was diluted with 50ml of dimethyl sulfoxide (DMSO) in a volumetric flask and sonicated for 10 min to extract ACV. This was again filtered by using Whatman filter paper no.1; one ml from this was further diluted with methanol up to 10 ml and absorbance measured at 251.3 nm using methanol as blank. After recording the absorbance the drug content and encapsulation efficiency were calculated. The readings were taken thrice and the average reading was taken for further calculation.

Amount of drug = Abs−intercept/ slope (∗10∗100) /1000

% Drug content = Calculated amount of drug total /amount of microspheres X 100

Encapsulation efficiency = Calculated drug content /theoretical drug content X 100

***In-vitro* Drug Release Studies**

The in vitro dissolution studies were carried using USP- 34 paddle type dissolution apparatus. 50mg ACV loaded microspheres were placed in a dialysis bag and introduced into100 ml dissolution medium of buffer solution pH 7.4 maintained at 37± 0.5 ºC at a rotation speed of 50 RPM. 1 ml of aliquots was withdrawn at predetermined time intervals and an equivalent volume of fresh medium was replaced to maintain sink condition. The aliquots were diluted and analyzed spectrophotometrically at 251.3 nm to determine the concentration of drug present. The readings were taken thrice and the average reading was taken for further calculation.

Accelerated stability studies

The above prepared samples were kept in sealed vials for 7 days at 40^{o}C and 75% RH.

FORMULATION OF GEL

Preparation of gel base from *Aloe Vera*

The mucilage or pulp of *A. vera* leaf, which is free from any resinous content (the dark red resin has to be drain out by holding the leaf upside for several seconds until the resin drips

out), has to be taken to prepare *A. vera* gel. Then the mucilage was washed repeatedly with pure water, since it is highly acidic finally washings with 0.1N sodium hydroxide (NaOH) solution increase the pH of Aloe pulp. By using a blender, the pulp is to be blended to obtain the juice. Then the juice is prefiltered for many times by using a cotton bed to remove any adhered rind. Then repeated subjection of the juice to the vacuum filtration produces a clear fluid. The Carbopol 934 (1%) is mixed with aloe juice to prepare *A. vera* gel by dispersion technique, were lump free mixture will be formed, and it allows free entrapped air upon standing. During the dispersion of juice to carbopol (jellifies under alkaline conditions), 0.5%w/w methyl paraben was added. Then 0.5 N NaOH solutions was added drop wise until to form a gel. After that required quantity of Nerolidol was added in *aleo-vera* gel and mixed properly in mortar pestle. Finally, the obtained gel was stored in air tight container to prevent any reactions.

Incorporation of microsomes containing drugs into the gel

Under certain atmospheric conditions, at 25 rpm, Microsomes containing drugs were mixed into the prepared *A. vera* gel by using an electrical mixer.

The formulations are varied for the concentration of the penetration enhancer (Nerolidol) from 1-5% as it is found to be safe within this range. Above 5% Nerolidol can be irritating to the skin.

Table 2: Design of formulations.

Formulation Code	Acylovir Microsphere	Nerolidol	Methyl paraben	Propyl paraben	*Aloe vera* gel
P-1	2.5g	1% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-2	2.5g	2% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-3	2.5g	3% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-4	2.5g	4% w/w	0.15% w/w	0.08% w/w	q.s. 50 g
P-5	2.5g	5% w/w	0.15% w/w	0.08% w/w	q.s. 50 g

Evaluation parameters of Microsomal Gel

Determination of pH: pH of each formulation was determined by using pH meter (pH meter Toshconcl 54+) which was calibrated before with buffer solutions of pH 4, 7 and 9.

Determination of Viscosity: Viscosity of each formulation was determined using Brookfield viscometer (Brookfield viscometer; type DV-II PRO) with spindle at room temperature and at 5, 10, 20, 50 and 100 rpm.

Drug content: 0.2 gm of the gel formulation (equivalent to 10 mg of drug) was taken in 100 ml volumetric flask which contains 20 ml of phosphate buffer pH 7.4 and sonicated for 15 minutes. Volume was made upto 100 ml. 1ml of above solution was further dilute to 10 ml by using phosphate buffer of pH 7.4.The resultant solution was subjected to UV spectrophotometric analysis at 251.3 nm and the absorbance was noted down.

Spreadability: To determine spreadability of the gel formulations, two glass slides of known standard dimensions are selected. Formulation whose spreadability to be determined was place on one slide and then other slide was kept over its top such that the gel is sandwiched between the two slides. The slides were pressed upon each other so as to displace any air present and the adhering gel was wiped off. The two slides were placed onto a stand such that only the lower slide is held firm by the one opposite fangs of the clampclips and allows the upper slide to slip freely over it by the force of weight tied Tie the 20 gm weight to the upper slide carefully. The time taken by the upper slide to completely detach from the lower slide was noted. The spreadability was calculated by using the following formula.

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

Where, Value s is Spreadability, m is the weight tied to the upper slides, l is the length of glass slide, and t is the time taken.

***In-vitro* permeation study of Optimized Gel Formulation by Drug Diffusion Method**

The diffusion of Acyclovir from gel formulations was studied through egg membrane and cellophane membrane using the Franz diffusion apparatus. The donor cell was filled with 300 mg of gel formulation (equivalent to 15mg of drug). The receptor compartment is filled by phosphate buffer having pH 7.4. The temperature of the receptor compartment was to be maintained at 37 ± 0.5^{o}C by using circulation of hot water through the jackets of Franz diffusion cell. The samples were removed at predetermined intervals at 0.5,1,2,4,6 hours and replaced immediately with equal volume of receptor solution to maintain sink conditions. The removed samples were analyzed at 251.4 nm on UV spectrophotometer.

Stability study

Stability study is performed for F5 as the formulation shows greatest drug release and hence can be termed as ‘best formulation’ from within those that are developed.

Stability study was carried out for 1 month, the formulation was kept in stability chamber at 40°C and at 75% relative humidity. After one month the formulation was checked for following parameters.

- Physical examination
- Drug content
- Diffusion study

RESULT AND DISCUSSION

Preformulation Study

Characterization of drug: The organoleptic properties of the received sample of Acyclovir were found crystalline odorless white powder that complied as mentioned in literature. Its melting point was observed at 256 to 258 °C with decomposition. Acyclovir was sparingly soluble in water but dispersible in these solution, soluble in 0.1N HCl and Phosphate buffer (pH 7.4) and freely soluble in ethanol and methanol.

UV spectroscopy

Determination of λ_{max} in 7.4 pH buffer

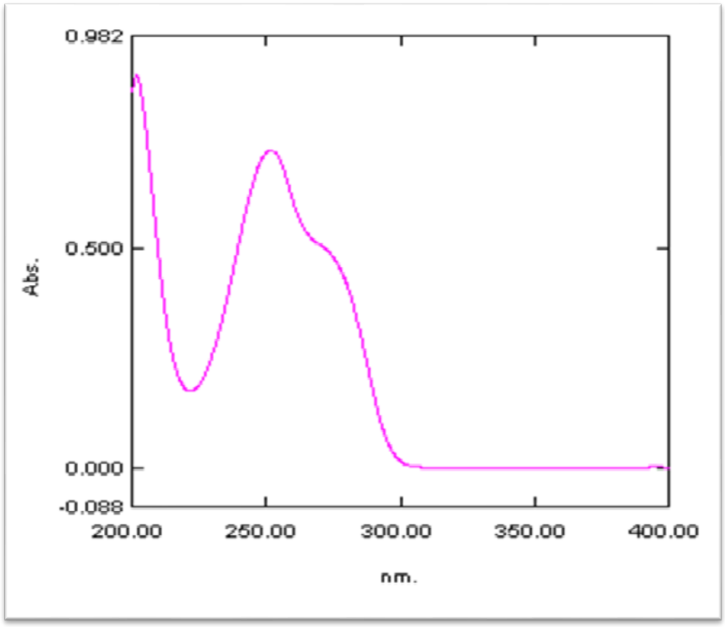

Figure 1: UV spectrum of Acyclovir in phosphate buffer of pH 7.4.

Wavelength of maximum absorbance (λ_{max}) for Acyclovir was observed at 251.3nm Phosphate buffer (pH 7.4)

Calibration Curve of Acyclovir in Phosphate buffer (pH 7.4)

Table 3: Absorbance values for Acyclovir in pH 7.4 buffer at 251.3nm.

Concentration (µg/ml)	0	4	8	12	16	20
Absorbance	0	0.242	0.443	0.645	0.878	1.074

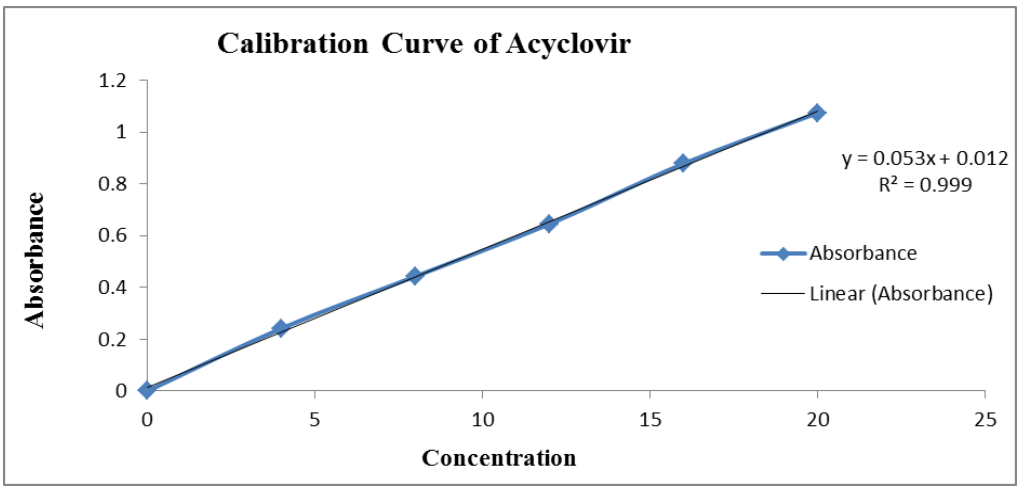

Figure 2: Calibration curve of Acyclovir in phosphate buffer (pH 7.4).

The calibration curve (figure 6.3) was found to be linear in the concentration range of 4-20 µg/ml having coefficient of regression value $R^2 = 0.999$ and line equation, y = 0.053x+0.012.

6.1.3 Fourier Transform Infrared (FTIR) analysis

Table 4: Interpretation of IR.

S. no.	Functional Groups	Groups	Type of Vibration	STD Range (cm^{-1})	Peak Observed (cm-1)
1.	Aromatic Ring	(- C - H)	Stretch	3150 - 3050	3103.50
		(= C - H)	bending	900 -690	864.15
2.	Alkane	(-C-H-)	Stretch	3000 - 2850	2860.42
		(-CH2-)	Bending	1465	1485.25
3.	Amine	(- NH2)	Stretch	3500 - 3100	3443.02
4.	Ether	(- C - O-)	Stretch	1300 - 1000	1107.16
5.	Alcohol	(O-H)	Stretch	3500 - 3650	3522.11
6.	Lactam Ring		Stretch		1712.75

Principal peaks for IR of Acyclovir are 1712, 1637, 1485, 1107 cm^{-1} matches with the standard spectrum for Acyclovir.

Formulation of Microsomes of Acyclovir

The ACV microspheres were formed due to the ionotropic gelation of the monovalent sodium alginate, an anionic polymer by the divalent Ca++ ions emerging from calcium chloride. The gelling was further enhanced due to the formation of an interpolymer polyelectrolyte complex

between the anionic alginate and cationic chitosan polymer. Thus the chitosan alginate polyelectrolyte complex retards the diffusion of the drug.

Table 5: Composition of ingredients Microsomes of Acyclovir.

Code	Acyclovir (%)	Sodium alginate conc. (% w/v)	Calcium chloride conc. (% w/v)	Chitosan conc. (% w/v)
P1	1	2	5	1
P2	1	4	5	2
P3	1	4	2.5	1
P4	1	2	2.5	2
P5	1	3	2.5	1

Characterization of the ACV Microsomes

Optical microscopy

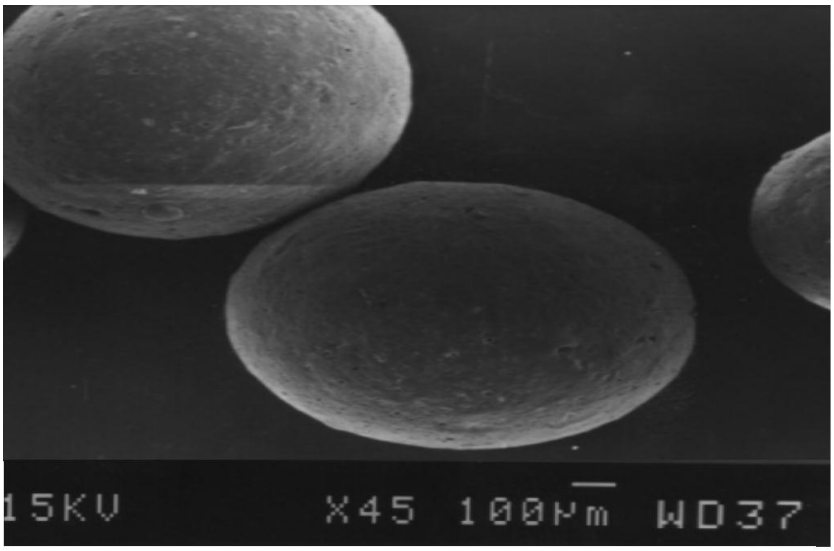

Figure 3: Surface morphology of optimized formulation.

Note: The shape was spherical with slightly elongated tips found in some microspheres.

Characterization of ACV Microsomes

Table 6: Results of characterization of ACV Microsomes.

Formulation	% Yield	Microsome Size (µm)	(%)Encapsulation	Swelling Index
P-1	89.73	568.65 ± 0.461	80.21 ± 0.25	3.52
P-2	86.49	715.57 ± 0.154	82.46 ± 0.45	3.04
P-3	94.22	636.12 ± 0.506	97.01 ± 0.85	3.64
P-4	83.54	751.82 ± 0.297	93.62 ± 0.75	3.14
P-5	87.34	585.65 ± 0.898	84.36 ± 0.45	3.28

Statistically significant difference among the values ($P < 0.0001$)

***In-vitro* Drug Release Studies**

Table 7: *in-vitro* cumulative % drug release from Microsomes.

Time (hrs)	P-1	P-2	P-3	P-4	P-5
0	0	0	0	0	0
1	07.44	09.00	10.56	09.60	07.20
2	14.14	18.45	17.12	19.77	14.49
4	21.91	27.89	25.98	28.49	22.63
6	29.33	36.99	34.23	39.02	29.44
8	36.86	45.48	41.29	49.31	37.46
10	44.88	55.41	48.46	58.88	44.75
12	51.69	67.25	57.20	67.61	51.57
16	60.31	77.66	67.97	77.29	58.15
18	66.76	87.10	75.97	87.82	65.57
20	75.50	89.71	84.83	88.99	73.34
24	81.23	92.82	94.39	90.90	79.79

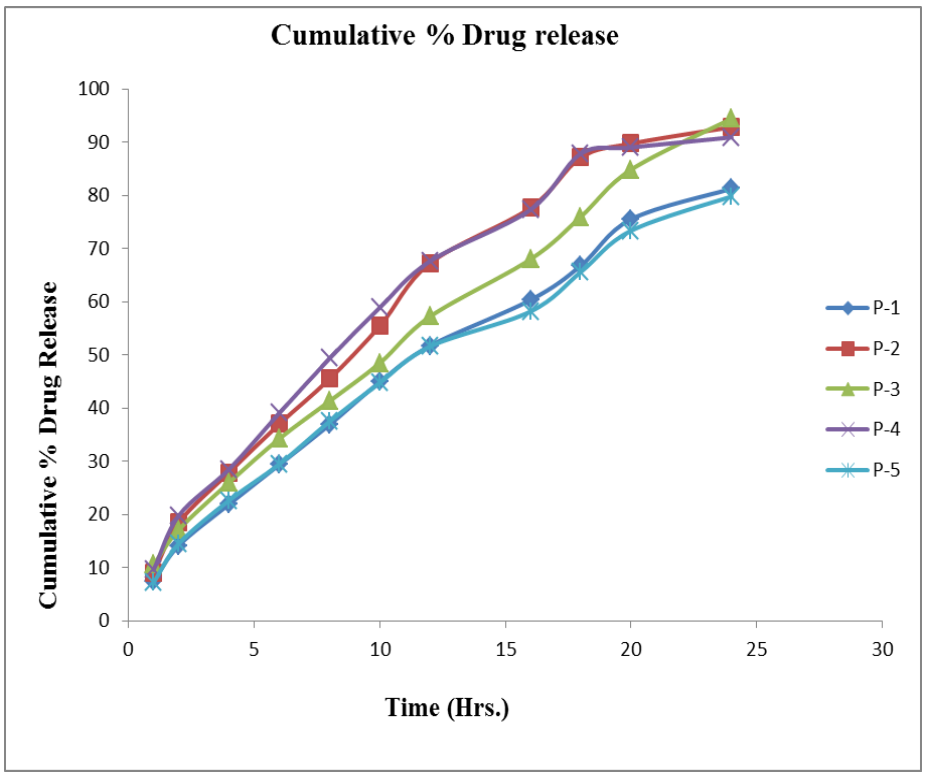

Figure 4: Cumulative % drug release of prepared Microsomes.

Table 8: R^2 values of all Microsome forulations.

Model	Equation	R^2
P-1	y = 3.350x + 7.057	R^2 = 0.980
P-2	y = 4.006x + 10.24	R^2 = 0.959
P-3	**y = 3.774x + 8.436**	**R^2 = 0.986**
P-4	y = 3.912x + 12.02	R^2 = 0.944
P-5	y = 3.249x + 7.601	R^2 = 0.976

Preparation of *aloe-vera* gel base using penetration Enhancer Nerolidol

Aloe-vera gel base using penetration Enhancer Nerolidol was prepared as designed formula described in method section.

Evaluation of Microsomal gel preparation

Determination of pH, viscosity, % Drug content, Spreadability

Table 9: pH of formulations.

Formulation code	pH	Viscosity (cps)	Drug content (%)	Spreadability (gm.cm/sec)
P-1	6.63	54703	98.92	12.66
P-2	6.63	52779	100.32	11.30
P-3	6.63	58767	99.40	11.23
P-4	6.61	54587	98.69	12.34
P-5	6.52	47770	100.35	12.69

Viscosity is an expression of the resistance of the fluid to flow; the higher the viscosity, the greater the resistance.

It is observed in reports that the viscosity of the formulations goes on decreasing as the rpm increases i.e an inverse relationship exists between the viscosity and the shear rate. From the figures it can be said that the formulations follow pseudo plastic behavior.

DIFFUSION STUDY

Diffusion study using cellophane membrane

Table 10: Cumulative amount of drug released Q (µg/cm^2) at different time intervals across cellophane membrane

Time (hr)	Q (µg/cm^2)						
	P-0	**P-1**	**P-2**	**P-3**	**P-4**	**P-5**	**Marketed**
0.5	170.3	288.49	173.56	129.04	253.94	504.00	142.01
1	329.42	601.25	573.00	619..05	540.69	828.03	235.90
2	590.58	931.99	780.50	628.04	777.79	1251.04	248.79
4	754.62	1221.0	871.13	801.00	929.70	1577.02	296.00
6	1065.80	1251.01	1395.01	1474.92	1522.89	1579.45	398.05

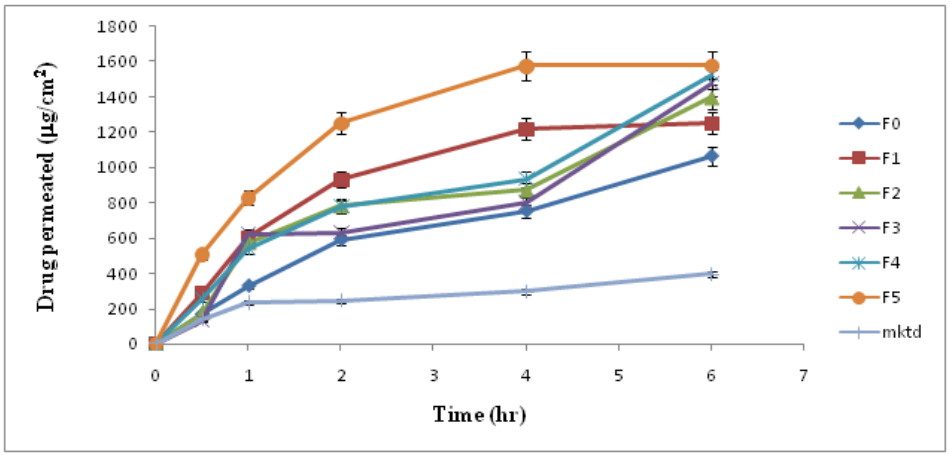

Figure 5: Plot of cumulative amount of drug release v/s time for different formulations of Acyclovir gel across cellophane membrane.

The release profiles were constructed by plotting the cumulative amount of Acyclovir diffused per unit membrane surface area (Q, µg/cm^2) versus time (hr). The steady state flux (Jss, µg/cm^2.hr) of Acyclovir was calculated from the slope of the plot using linear regression analysis.

Table 11: Results of drug diffusion parameters across cellophane membrane.

Formulation code	Q_6 (µg/cm^2)	Jss (µg/cm^2 · hr)
P-0	1065.80	177.05
P-1	1251.01	198.40
P-2	1395.01	202.00
P-3	1474.92	214.50
P-4	1522.89	225.10
P-5	1579.45	240.9
Marketed	398.05	166.3

It is observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

Using egg membrane

Table 12: Cumulative amount of drug released (Q) µg /cm^2 at different time intervals across egg membrane.

Time (hr)	Q (µg/cm^2)						
	P-0	**P-1**	**P-2**	**P-3**	**P-4**	**P-5**	**Marketed**
0.5	231	711.9	485.8	786	523.97	685.44	369.37
1	336.1	1046	1166.08	877.99	1119.01	869.99	534.28
2	363.01	1164.98	1210	1027.03	1391.06	1512.96	571.02
4	522.9	1286	1298.04	1314.88	1428.01	1552.08	539.78
6	695.2	1303.24	1376	1425.22	1474.98	1564.42	609.07

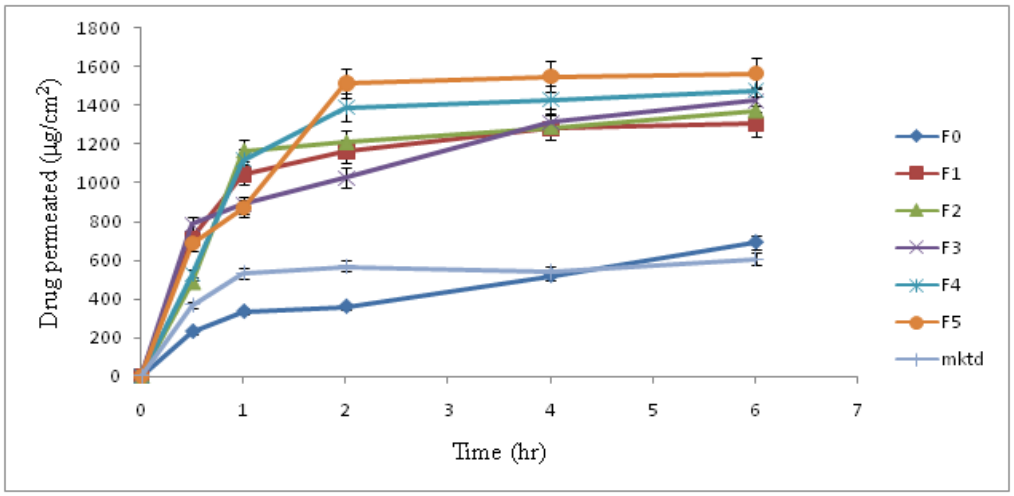

Figure 6: Plot of cumulative amount of drug release v/s time for different formulations of Acyclovir gel across egg membrane.

The release profiles were constructed by plotting the cumulative amount of Acyclovir diffused per unit membrane surface area (Q, **µ**g/cm^2) versus time (hr). The steady state flux (Jss, **µ**g/cm^2. hr) of Acyclovir was calculated from the slope of the plot using linear regression analysis. The permeability coefficient (Kp) and enhancement ratio (ER) are calculated using following equations:

$$Kp = \frac{Jss}{C}$$

Where, Jss is flux at steady state and C is the initial concentration of Acyclovir in the donor compartment:

$$ER = \frac{Kp\ with\ pretreatment}{Kp\ without\ pretreatment}$$

Table 13: Results of drug diffusion parameters across egg membrane.

Formulation code	Q_6 (µg/cm^2)	Jss (µg /cm^2 · hr)	Kp (x10^{-3})	ER
P-0	695.20	97.05	6.44	
P-1	1303.24	159.56	10.13	1.68
P-2	1376.00	178.39	10.99	1.89
P-3	1425.22	182.32	12.15	1.90
P-4	1474.98	199.17	13.30	2.02
P-5	1564.42	220.66	14.48	2.29
Marketed	609.07	67.42	4.3	

Q6= Cumulative amount permeated at 6 hr; Jss= Steady state flux;Kp= Permeability coefficient; ER= Enhancement ratio

It is observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

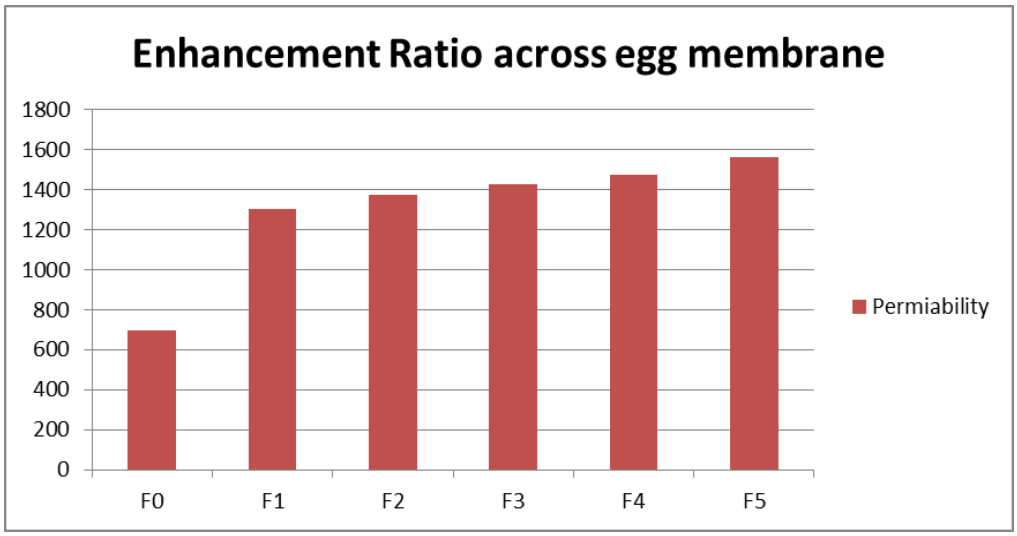

Figure 7: Effect of Nerolidol in terms of Enhancement Ratio across egg membrane.

Stability Study

Physical examination: No visible change in the formulation was observed as compared to control formulation.

Determination of drug content

Table 14: Drug content for stability testing.

Drug content after stability	Drug content before stability
98.01	100.33

Diffusion study

Table 15: Results of drug diffusion parameters across egg membrane for stability testing.

Time (hr)	Q_6 (µg/cm^2)	Jss (µg/cm^2 · hr)
0.5	658.11	220.33
1	849.02	
2	1495.56	
4	1545.15	
6	1551.65	

The cumulative amount of drug permeated at 6 hour (Q_6) and the steady state flux (Jss) for the formulation were found to be 1551.65µg/cm^2 and 220.33µg/cm^2·hr respectively. No significant changes were observed in the formulation after its stability testing.

DISCUSSION

This novel drug delivery system promotes the importantly ease and convenience of administration, deliverance of accurate dose as well as to prolong residence time of drug in contact with skin, that problems generally encountered in semisolid dosage forms.

During the Preformulation studies it is found that the organoleptic properties of acyclovir comply as reported. White crystalline, tasteless, odorless, substance was sparingly soluble in water but dispersible in these solution, soluble in 0.1N HCl Phosphate buffer (pH 7.4) and freely soluble in ethanol and methanol. Acyclovir was observed melting point at 256 ^{0}C. λ_{max} was determined in Phosphate buffer (pH 7.4) solvent at 251.3 nm. Standard calibration curve was prepared using concentration range 04-20µg/ml and linearity equation as y = 0.053x+0.012 with R^2 = 0.999. Drug Acyclovir was also compatible with used excipients. It is physically stable and chemically stable as observed by FT-IR spectra.

Five different formulations were prepared all the sodium alginate solutions were prepared by dissolving the respective amounts in distilled water. To the sodium alginate solutions, 1% w/v of acyclovir was added under homogenization for 5 min to yield smooth dispersions of acyclovir in sodium alginate solutions. The chitosan solutions (1% w/v or 2% w/v) were prepared in 5% v/v aqueous acetic acid. To the chitosan solutions, respective amount of calcium chloride was added. The acyclovir–alginate dispersions were taken into syringe fitted with 0.45 mm needle and dropped at the rate of 1ml/min into chitosan-calcium chloride solutions stirred at 100 RPM at room temperature to yield opalescent beads. The microspheres were allowed to harden for another 2 h, filtered, washed thrice with distilled water and kept for drying at 40 ºC in an oven for 24 h.. Dried microcapsules were subjected for Evaluation were found yield between 83.54 to 94.22 %, mean particle size between 568.65 to 751.82 µm and % encapsulation between 80. 21 to 97.01% and swelling Index between 3.04 to 3.64, shape was spherical with slightly elongated tips found in some microspheres. All micrsome formulations give more than 79 % drug release in steady way it was decided that all formulations were incorporated in gel preparation. On the basis of various parameter of evaluation of microsomal gel formulations, pH of all formulations were adjusted about 6.63, viscosity at 5 rpm was maximum at 58767 cps, % drug content between 98.69 to 100.35% and Spreadability maximum 12.69 gm.cm/sec**.** All formulation (P-1 to P-5) with a blank formulation (P-0 only pure drug) and a marketed formulation were subjected for diffusion study with cellophane membrane and egg membrane. Cumulative amount of drug

released Q (µg/cm^2) at different time intervals and steady state flux (Jss, µg/cm^2.hr) were obtained. Also permeability coefficient (Kp) and enhancement ratio (ER) are calculated and found **P-5** has highest enhancement ratio (ER) 2.29 and **P-4** has 2.02. Also it was observed that the flux and permeability of Acyclovir goes on increasing as the concentration of Nerolidol increases.

Accelerated Stability studies for 30 days was performed with optimized Microsomes batch **P-5** for physical examination observed no visible change, % Drug content after stability and before stability were 98.01 and 100.33 respectively. The cumulative amount of drug permeated at 6 hour (Q6) and the steady state flux (Jss) for the formulation were found to be 1551.65µg/cm^2 and 220.33µg/cm^2.hr respectively. No significant changes were observed in the formulation after its stability testing.

CONCLUSION

Microsomes of acyclovir were prepared using the sodium alginate solutions, 1% w/v of acyclovir, the chitosan solutions (1% w/v or 2% w/v) were prepared in 5% v/v aqueous acetic acid. To the chitosan solutions, respective amount of calcium chloride was added. The acyclovir–alginate dispersions were taken into syringe fitted with 0.45 mm needle and dropped at the rate of 1ml/min into chitosan-calcium chloride solutions stirred at 100 RPM at room temperature to yield opalescent beads. Because of acyclovir was sparingly soluble in water, so, the drug requires a novel drug delivery system which can provide enhanced solubility, an extended period of time and improve absorption via skin. Nerolidol was used as permeability enhancer of Acyclovir. Microsomes were characterized for compatibility study, particle size and shape, % entrapment, *in-vitro* drug release. Due to their matrix character, these drug delivery systems showed good enhanced solubility and sustained release, required for bioavailability and therapeutic activity. Acyclovir also causes irritation on skin, *aloe-vera* was used in preparations which reduced irritation. Major advantages of the system include ease of preparation, good enhanced solubility, high % encapsulation efficiency and sustained drug release. From this study, it was concluded that microsomes of Acyclovir were offers enhanced solubility and continuous release of the medicament and bioavailability of the drug and subsequent efficacy is improved.

CONFLICTS OF INTEREST

There are no conflicts of interests.

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